QUALITY ASPECTS OF CULTURED GULF OF MEXICO STURGEON *ACIPENSER OXYRHYNCHUS DESOTOI* AS A POTENTIAL FOOD COMMODITY

By

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IT

To my beloved daughter, Carolina, for her love and patience throughout this work To my mother, Beatriz, for her love encouragement, and commitment in helping my dreams come true.

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Abstract of Dissertation Presented to the Graduate School
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Hormone-induced ovulation and spawning of Gulf sturgeon occurred in 1989 at a hatchery located on the Suwannee River in Florida. During subsequent years, the University of Florida in conjunction with the US Fish and Wildlife Service and Caribbean Conservation Corporation, produced about 60,000 fry for fish culture programs. The first objective of this study was to investigate rigor mortis in Gulf sturgeon. A video capture method was developed to monitor rigor mortis in whole fish. The method was able to determine onset and resolution of rigor in Gulf sturgeon, as well as to provide insight into pattern of rigor development over time. The second objective was to determine the influence of diet type on the quality (defined as rigor, color, appearance, taste and mouthfeel, composition, and fatty acid profile) of Gulf sturgeon fillets as a food commodity. Three commercial diets with different fat contents were used during feeding trials at the Gulf sturgeon demonstration farm facility (University of

Florida, Blountstown, FL). Fish were received live at the Food Science and Human Nutrition Department at the University of Florida during the summer of 1999 (Gainesville, FL). Sensory panels to evaluate the taste and appearance, color studies over storage, proximate analysis and fatty acid composition were conducted to determine the most suitable diet. Feeding Gulf sturgeon with trout diet, containing the highest percent fat, resulted in the highest ratio of ω3/ω6 fatty acids found in sturgeons included in the study. However, trout diet imparted a yellow-orange coloration to the fish muscle, which was unappealing to consumers. Feeding Gulf sturgeon with catfish diet, containing the lowest fat content, results in a poor fatty acid profile which is markedly different from the fatty acid profile of wild sturgeon muscle. Fillets from sturgeons fed hybrid bass diet performed well on consumer sensory panels for taste and appearance, and the fatty acid profile was similar to the profile of fillets from fish fed trout diet. Nonetheless, there was no detrimental pigmentation of the muscle due to feed. It was concluded that among the diets investigated, hybrid bass is the most suitable to feed cultured Gulf sturgeons.

CHAPTER 1
INTRODUCTION

Importance of Sturgeon Species in the United States

Sturgeons have been exploited and considered a valuable commodity since ancient times. By the beginning of the twentieth century several sturgeon species were practically extinct or reduced to small populations. Even though water pollution and habitat destruction greatly contributed to stock depletion, over-fishing is especially threatening to the family *Acipensedae* (Stetzar and Dore, 1993). Worldwide productions of sturgeon meat and caviar have drastically declined over the past 30 years. From 1983 to 1992, sturgeon landings have decreased from 27 thousand metric tons (MT) to 14 thousand MT, and caviar production decreased form 25 hundred MT to about 900 MT (Wedic and Fadal, 1997). Political difficulties, such as the disappearance of cartel arrangements between the former Soviet Union and Iran, have led to an unmanageable increase in fishing activities in the Caspian Sea. This traditional sturgeon producing area is now under the jurisdiction of seven political entities. Lack of guidelines and proper arrangements are placing the vitality of the sturgeon fisheries in the Caspian Sea in jeopardy (Doroshov et al., 1995).

In 1913, with the breakthrough in artificial propagation of sturgeon species, Russia launched a hatchery program for ranching (repopulation of the rivers and seas) and later for farming (aquaculture).

In 1991, the production of farmed sturgeons in Russia exceeded 500 metric tons (Sternin and Dore, 1993) The success of sturgeon mitiganon programs in Russia proved that it is possible to help renew wild stocks, as well as to culture sturgeon species for commercial purposes

In the USA, artificial propagation of White sturgeon (*Ancipenser transmontanus*) led to a profitable commercial aquaculture industry in California Currently, White sturgeon is commercially grown and highly priced for its caviar, fresh and smoked meat (Chapman et al., 1997, Barsh, 1997) Hormone-induced ovulation and spawning of Gulf of Mexico sturgeon took place for the first time in 1989 at a hatchery located on the Suwannee River in Florida (US Fish and Wildlife Service 1995) During subsequent years, the University of Florida in conjunction with the FWS (US Fish and Wildlife Service) and CCC (Caribbean Conservation Corporation) produced about 60 000 fry for fish culture programs (US Fish and Wildlife Service, 1995) Like other sturgeon species, there is great potential for commercialization of Gulf of Mexico Sturgeon raw and smoked white muscle as well as caviar, in the domestic and international market.

Importance of Monitoring Rigor Mortis in Aquaculture Fish Species

Rigors mortis from the Latin stiffness of death, can impact the quality of fish muscle. Establishing and monitoring the conditions under which fish undergo this phenomenon is imperative to assure safety and quality of the food product.

It is known that most fish species undergo rigor within 48 hours after death Therefore, when fish arrive at the processing plant, rigor is normally over This is especially true for fish caught in deep waters, because fishing vessels can take several weeks to return to shore. In this scenario fish undergo rigor mortis on ice in the fishing

vessel. Thus, fish processors are generally spared from the need to address this problem. Nonetheless, fish harvested in an aquaculture facility requires guidelines for handling the product between death and commercialization, whether as whole fish (gutted) as butlers or as dressed fillets.

It is important to develop an effective, non-destructive, fast and low cost methodology to monitor rigor mortis in cultured fish. This will allow accurate determination of the time frame and optimal conditions for a specific species to undergo rigor with minimal quality loss.

Importance of Diet Composition in Aquaculture Fish Species

Commercial diets are normally formulated to answer the needs of a fish family or a specific species. To ensure maximum profitability, an aquaculture facility has to choose a diet that supplies proper nutritional content for optimal growth. In addition, it has to be economically viable to support profitability of the business and it has to be commercially available to ensure a steady supply. Finally, the chosen diet cannot cause detrimental effects in the final product such as low production yields, improper coloration, off-flavors, low nutritional value or textural changes.

Presently, many commercial diets for fish, including trout, catfish, salmon and bass, are available for purchase. However, there are no commercial diets specifically formulated for any sturgeon species. It is of prime importance to the rising aquaculture industry in Florida to find a suitable diet for cultured Gulf of Mexico Sturgeon.

Objectives

The overall objectives of this study were to investigate the process of rigor mortis, to determine yields for commercialization, and to investigate the influence of three different commercial diets on quality attributes of Gulf of Mexico Sturgeon (*Acipenser oxyrynchus desotoi*)

The specific objectives of this work were the following

1. To set specific guidelines for handling Gulf sturgeon after death and prior to processing,
2. To develop a fast, non-destructive, low cost and reliable methodology to determine rigor mortis in Gulf sturgeon,
3. To set specific guidelines to filleting Gulf sturgeon with maximum yields
4. To determine proximate composition and its variability between and within Gulf sturgeon fillets,
5. To investigate the influence of three commercial diets on sensory properties, coloration, proximate composition and fatty acid profile of Gulf sturgeon raw and smoked fillets.

CHAPTER 2
LITERATURE REVIEW

Sturgeons

Sturgeons are one of the oldest and most primitive fishes, being actually considered "living fossils" since fossil acipenseriforms are known from the Jurassic period (Birstein 1993). It is believed that sharks are distant ancestors, and that around 250 million years ago (*Homo sapiens* appeared 40 thousand years ago) spontaneous genetic mutation created the family of *Acipenseridae* (Sternin and Dore, 1993). Since then, these gentle toothless fish that inhabit northern hemisphere waters have managed to survive many geological cataclysms (Sternin and Dore, 1993). However, it has been clearly harder for them to survive over fishing, water pollution, habitat destruction and other man made interferences in their native environment (Sternin and Dore, 1993). Overfishing is especially threatening to this family because they mature rather late and they can take up to 15 years to breed for the first time (varies greatly between populations) (Sternin and Dore, 1993, Birstein, 1993). In addition, they can take long periods of time between spawning (Birstein, 1993). Sturgeons migrate up freshwater rivers to spawn and prefer shallow spawning areas, where they became more vulnerable to man (Sternin and Dore, 1993). Therefore, a large number of sturgeon species are reduced to small populations and others are very close to extinction (Sternin and Dore, 1993).

Sturgeons (*Ancipenseridae*) and paddlefish (*Polyodontidae*) are the main representatives of the *Chondostei* (infra class) primarily known as producers of black caviar (Birstein 1993). The roe of sturgeon is considered a delicacy, commercialization is extremely profitable and market price of good quality caviar has always been extremely high. Sturgeon flesh has also excellent commercial value and it is often smoked. Sturgeon has been exploited and considered a valuable commodity since ancient times and coins depicting sturgeons were minted around 600 B.C. from ancient Carthage (Sternin and Dore 1993). In addition, Aristotle (350 B.C.) cited the use of sturgeon swim bladders to produce isinglass, a semitransparent gelatinous substance used in jellies, wine and beer clarification, special cements and glue (Barkuloo, 1988). It is believed that Alexei Michailovich (the second Romanov Tsar) was the first to recognize the value of caviar export trade in 1675 (Sternin and Dore, 1993). Since then, caviar and also isinglass were commercialized on a regular basis (Sternin and Dore, 1993). As a consequence, natural stocks all over the world were rapidly depleted or dropped drastically in a short period of time.

Sturgeons inhabit the inland and coastal waters from the Arctic Circle to the subtropics (Doroshov 1985). Their geographic ranges encompass all major river watersheds in North America, Europe and Asia (Table 2.1). Originated in inland fresh or estuarine waters, these fish normally prefer temperate waters (Doroshov, 1985). Even though *chondrosteans* were very abundant since the Upper Cretaceous period, the number of species and populations greatly decreased (Doroshov, 1985). Currently, just two species of paddlefish (*Polyodontidae*) and approximately 28 species and subspecies of sturgeon (*Ancipenseridae*) can still be found in nature (Doroshov 1985).

The sturgeon family consists of four genera. Belugas (*Huso*), Sturgeon (*Acipenser*) Shovelnose sturgeons (*Scaphirhynchus*) and False Shovelnose sturgeons (*Pseudoscaphirhynchus*) (Sternm and Dore, 1993) Table 1-1 shows all currently recognized species and subspecies together with their corresponding English name, other common names, status and distribution.

Table 2-1 The Sturgeon family

Species and Subspecies	English Names and Other Names	Distribution	Status
Acipenser baeri	Siberian sturgeon	Main Siberian rivers (Ob, Irtysh Yenisei Lena, Kolyma)	Insufficiently known[a] artificially propagated[b,c]
A. baeri baicalensis	Baikal sturgeon	Lake Baikal (Siberia)	Endangered[d]
A. brevirostrum	Shortnose sturgeon	Rivers estuaries, and the sea along east coast of North America from Indian River to St.John River	Vulnerable[e], threatened[f]
A. dabryanus	Yangtze sturgeon	Yangtze River system	Close to extinct[a] endengereds
A. fulvescens	Lake sturgeon	The Great Lakes and several lakes of southern Canada	Vulnerable[e] threatened[f]
A. gueldenstaedti	Russian sturgeon= Osetra[g] Kura sturgeon[g]	Black, Azov and Caspian Seas and rivers entering into them	Vulnerable[e], artificially propagated[b,c]
A. gueldenstaedti colchicus[g]	Black Sea sturgeon[g]	Caspian Sea, Black Sea and rivers entering into them[g]	
A. medirostris	Green sturgeon	Pacific coast of North America from Aleutian Islands and Gulf of Alaska to Ensenada, Mexico	Vulnerable[e]
A. multiscuatus[g]	Japanese sturgeon[g]	Information not available	
A. mikadoi	Shakhalin sturgeon	Pacific Ocean from the Amur River to northern Japan, Korea, and Bering Sea, Tumpin (Datta) River	Intermedrate[a]

Table 2-1 continued

Species and Subspecies	English Names and Other Names	Distribution	Status
A. naccarii	Adriatic sturgeon	Adriatic Sea, Po and Adige Rivers	Vulnerable[a]
A. nudiventris	Thorn sturgeon*, Ship sturgeon	Caspian Sea and rivers entering to it	Endangered[b], artificially propagated**
A. oxyrhinchus desotoi	Gulf sturgeon, Gulf of Mexico sturgeon	Gulf of Mexico and northern cost of South America	Threatened[b]
A. oxyhinchus oxyrhinchus	Atlantic sturgeon	Several rivers, estuaries, and the sea along east const of North America from the Hamilton Inlet (Labrador) to the St John River (Florida)	Vulnerable[a], special concern[b]
A. persicus (also found classified as a subspecies *Acipenser gueldenstaedti persicus*)*	Persian sturgeon	Caspian and Black Seas and rivers entering into them	Endangered[b]
A. ruthenus	Sterlet	Drainages of the main rivers entering the Caspian and Black Sea	Endangered[b], artificially propagated**
A. schrenckii	Amur sturgeon	Amur River System	Vulnerable[a]
A. sinensis	Chinese sturgeon, Sagami sturgeon*	Yangtse River system (China)	Endangered[b]
A. stellatus	Stellate sturgeon, Sevruga*, Star sturgeon*	Caspian, Azov, Black and Aegean Seas and rivers entring into them	Intermediate[a], artificially propagated**
A. sturio	European sturgeon, Baltic sturgeon, Atlantic sturgeon, Common sturgeon	Baltic, Estern North Atlantic, Mediterranean, Black Sea and rivers of Western Europe	Close to extinction[a], endangered[b]
A. transmontanus	White sturgeon	Pacific coast of North America from the Gulf of Alaska to Baja California	Vulnerable[a]
Huso dauricus	Kaluga, Great Siberian sturgeon*	Amur River system	Rare[a]
H. huso	Beluga, Giant sturgeon, Hausen*	Caspian, Black and Adriatic Seas and rivers entering into them	Vulnerable to extinct[a], art ficially propagated**, Azov Sea population is extinct*

Table 2-1 continued

Species and Subspecies	English Names and Other Names	Distribution	Status
Hybrid of *H. huso (female)* and *A. ruthenus (male)**	Bester* Hybrid sturgeon*	Farmed sturgeon species of commercial importance in Russia and Kazakhstan (First bred by I. Bastev in 1952)*	
Pseudoscaphirhynchus fedtschenkoi	Syr-Darya shovelnose Syr Dar shovelnose	Syr Dayra River (Kazakhstan)	Extinct[a] ,endangered[b]
P. hermanni	Dwarf sturgeon* Little shovelnose sturgeon* small Amu-Dar shovelnose	Amu Dayra River (Uzbekistan)	Extinct[a] endangered[b]
P. kaufmanni	Amu Darya sturgeon* large Amu-Dar shovelnose	Amu-Dayra River (Uzbekistan)	Close to extinction[a] endangered[b]
Scaphirynchus albus	Pallid sturgeon	Missouri and Mississippi River basins	Endangered[b]
S. platorynchus	Shovelnose sturgeon	Missouri and Mississippi River basins	Endangered[b]
S. suttkusi	Alabama sturgeon	Mobil basins in Alabama and Mississippi River basins	Endangered[b]

a= categories given in the IUNC system

b= categories given in the U.S. Office of Endangered Species system

* Information gathered from "Caviar: The resource Book" (Sternin and Dore, 1993)

** Artificially propagated designates species whose natural reproduction is limited. These are artificially bred and then released into their natural habitat.

Adapted from: Birstein, 1993

Life History

Longevity is a remarkable characteristic of many sturgeon species: as an example it can be pointed that Beluga can live over 100 years (Doroshov, 1985). It seems that this is mainly due to the low natural mortality in the adult phase, especially for the fast growing species (Doroshov, 1985). Low natural mortality of adult sturgeons is primarily attributed to absence of natural predators (Doroshov, 1985). The slow-growing species normally have a shorter life span (around 10 to 20 years), although some species can live

up to 50 years such as the Siberian sturgeon (Doroshov 1985) In addition, some fast-growing sturgeon species can reach an extremely large body size In 1769 it was reported that a Beluga caught in the Ural River (Guryev) weighted 2,000 kg and measured 6 3 meters long (Sternin and Dore, 1993) At the end of the 1800s and beginning of the 1900s White sturgeons were caught in several rivers in US and reported weights ranged up to 800 kg for one single fish (Sternin and Dore, 1993) Unfortunately, the intense commercial exploitation has cause of a great reduction in the mean size of all sturgeon stocks (Doroshov 1985) It seems that the smallest sturgeon are the land-locked riverine species such as Sterlet and Shovelnose Average weight for these species ranges from 300 g to 4 kg, and maximum body weight normally does not exceed 25 kg (Doroshov, 1985)

Migration is another important characteristic of sturgeon species Even though they are said to be anadromous just a small number of species are truly anadromous (Doroshov 1985) These species that migrate into fresh water just to spawn include the Green sturgeon and the North American and European species of Atlantic sturgeon (Doroshov 1985) Some other species can be encountered in full-strength seawater such as Beluga, Kaluga and White sturgeon, however they have a preference for brackish water estuarine environments (Doroshov 1985) A second group of sturgeon species, and the most abundant ones, can be classified as semi-anadromous (Doroshov, 1985). Some examples are Russian sturgeon, Sevruga, Schip and White sturgeon These species live in the estuaries of large rivers or brackish water lakes (Caspian and Aral Seas) and migrate up river to spawn (Doroshov, 1985) It seems that many of these species are euryhaline and can resist a wide range of water salinity Apparently, some of these

species can spend all their life cycles in fresh water, such as Russian sturgeon in the Volga River and Schip in Lake Balkhash (Doroshov, 1985). There is still a third group of entirely freshwater sturgeon species. They can inhabit freshwater lakes and go up river just to spawn such as Lake sturgeon and Siberian sturgeon, or they spend their entire life in riverine environment such as Sterlet, Shovelnoses and Shortnose sturgeon (Doroshov, 1985).

One of the biggest problems, in terms of maintaining sturgeon populations within acceptable numbers, is the fact that all sturgeon species require a much longer time to reach sexual maturity than modern teleost fish (Doroshov, 1985). The median age of fish at their first spawning greatly varies among different species, but most stocks average between 10 to 20 years (Doroshov 1985). Apparently the fastest sexual maturation occurs in the small riverine Sterlet (4 to 8 years) and the slowest in the large anadromous species or species living in harsh climatic conditions such as the Siberian sturgeon that takes around 25 years to spawn for the first time (Doroshov 1985). It is important to mention that sexual differentiation also occurs rather late in many sturgeon species. Nonetheless, gonadal development appears to be better related to size than to age of the fish, once it was found that different stocks of one species exhibit almost twofold difference in median age of first sexual maturation (Doroshov 1985).

All species of sturgeon are iteroparous and spawn several times during their lives (Doroshov, 1985). In addition, generally sturgeons are very fecund, meaning that they produce a large number of eggs (Sternin and Dore, 1993). Beluga fecundity can range up to 3 million eggs (the record is thought to be 8 million) and European sturgeon up to 6 million eggs (Sternin and Dore 1993). Regardless, the annual fertility of sturgeon is

actually quite low due to the fact that intervals between spawning can be as long as 8 years (Sternin and Dore, 1993) Therefore, the reproductively active population does not exceed 10-20% of the adult stock in a given year (Doroshov, 1985)

Finally it can be pointed out that sturgeons are carnivores. Sturgeon species are similar in their feeding habits with a few exceptions (Doroshov, 1985) They have a protruding mouth and are generally described as 'bottom dwellers' The lips, barbels and snouts are equipped with numerous sensory receptors, which they use to identify their prey (Doroshov 1985) Most species are opportunistic feeders eating mollusks, worms, crustaceans and fish (Doroshov 1985)

Sturgeon Culture

In 1675 Pastor Hesselius wrote in German the first document warning about sturgeon over fishing (Sternin and Dore, 1993) Unfortunately it was not until severe depletion of the sturgeon stocks all over the world took place that laws and regulations started to prevent catch of undersized fish, to determine fishing areas and timed of fishery openings and to permit fishing gears (Sternin and Dore, 1993) One of the main causes of the sharp decline in sturgeon population in the Caspian Sea was marine sturgeon fishing (Sternin and Dore, 1993) At that time, not just the sexually mature fish returning to the rivers were caught, but the whole population was fished, greatly decreasing the number of spawners. The Beluga's share of the total catch dramatically declined in a short period of time (Sternin and Dore, 1993) As early as in the beginning of the 1900s the commercial fishery in the Delaware River (Pennsylvania) was almost depleted Commercial fisheries in the Sacramento Columbia, Snake and Fraser Rivers shared same fate (Sternin and Dore, 1993).

In Europe the results from over fishing were no different than anywhere else, and sturgeons from rivers such as the Danube, Rhine, Gironde, and Elbe were no longer available by the beginning of the 20th century (Sternin and Dore, 1993). Nevertheless, the demand for caviar did not decrease. The prices started to rise rapidly, and profitability of caviar business was beyond expectations. In the last twenty years of the nineteenth century, prices of caviar rose by 20 times (Sternin and Dore, 1993). Nowadays there are still some small sturgeon populations in the rivers Danube and Gironde, but it is very unlikely that sturgeon populations will return to the river systems of the Rhine, Elbe or Oder (Steffens et al., 1990). Today, Russia is one of the few countries where sturgeons are still economically significant, accounting for 80-90% of the total world catch (Steffens et al., 1990). Currently, all countries that still have remaining reservoirs of sturgeon species have severely restricted or totally prohibited commercial and sport fishing of sturgeon species (Sternin and Dore, 1993). Notwithstanding, sturgeon resources kept declining for numerous reasons such as loss of spawning grounds, water pollution and illegal fishery (Sternin and Dore, 1993).

In order to guarantee a more steady supply of caviar and maintain such a profitable business, the Russians started research into the possibility of artificially propagating sturgeon species. In 1869, Ovsyankov managed to incubate Sterlet eggs (Sternin and Dore, 1993) in Russia. Green achieved the first successful incubation of Atlantic sturgeon eggs in the US in 1875 (Doroshov et al., 1988). Several experiments in sturgeon culture were carried out in the US, including an attempt to artificially propagate short nose sturgeon in the Delaware River (Meehan, 1910). After 1920 this interest in researching this area faded in the US (Doroshov et al., 1988). The Russians kept working

towards the development of hatching and rearing technologies for sturgeon species (Sternin and Dore 1993) The breakthrough took place in 1913 with the introduction of a procedure that eliminated adhesiveness of the eggs by washing them with a suspension of silt (Sternin and Dore, 1993) Since then Russian scientists accomplished several achievements, and the most important were induced spawning, surgical removal of eggs (mortality rates were just 20 to 30%) and hybridization (Doroshov, 1985, Sternin and Dore, 1993) Hybridization turned out to be very important after the success achieved by the inter-generic hybrid of great Beluga sturgeon and the Sterlet (Doroshov, 1985) This fish, named Bester, grows as fast as the anadromous Beluga but matures as quickly as the freshwater Sterlet By the age of 9 to 12 years old it can weigh up to 17 kg, and yield up to 4 kg of caviar Moreover, subsequent generations from Bester proved the stability of this hybrid species (Sternin and Dore 1993)

There are two major ways to propagate sturgeons- ranching and farming. The first consists of hatching and rearing 2 to 3 gram fry artificially and then releasing them into the natural environment. After 10 to 15 years the fish can be harvested weighting 4 to 5 thousand times more than its weight at the time of release (Sternin and Dore, 1993) Even though the overall rate of return in the Caspian Sea is still low (approx 1%), there are about 20 rearing facilities in Russia producing millions of fry of all major commercial species such as Beluga, Russian sturgeon Sterlet, Sevruga and Ship (Sternin and Dore, 1993)

Farming started in Russia as an alternative method to ranching The major difficulty is that the eggs have to be taken before they reach the "running stage When eggs are taken at this stage the mortality rates are extremely high, so eggs are normally

obtained just once (Stemin and Dore, 1993). Its success encouraged researchers in the US to reinitiate studies of sturgeon propagation in the late 70's (Doroshov et al , 1988) In the late 80's, Doroshov and his team at the University of California at Davis develop hatchery technology for White sturgeon (Doroshov et al , 1988) Presently efforts on the West Coast, in the Great Lakes area, and on the East Coast have accelerated both hatchery and fisheries research The ultimate goal is to rebuild US sturgeon populations and stimulate commercial sturgeon aquaculture (Doroshov et al., 1988)

It can be pointed out that commercial sturgeon farming is now available in several countries such as France, Italy, Spain, Germany, Hungry and the US In addition, in 1991 the production of farmed sturgeons in Russia already exceeded 500 tones (Stemin and Dore, 1993) World production of farmed sturgeon is expected to reach thousands of metric tons per year very soon Moreover, processing caviar from farmed sturgeons at a reasonable cost is likely to become routine in the near future (Stemin and Dore, 1993)

Gulf of Mexico Sturgeon

Taxonomy

Class: *Osteichthyes*

Order: *Acipenseriformes*

Family *Acipenseridae*

Genus *Acipenser*

Species. *oxyrinchus*

Subspecies *desotoi*

Description

Acipenser oxyrinchus desotoi is commonly known as Gulf of Mexico sturgeon, but other colloquial names are Gulf sturgeon, Atlantic sturgeon, Gulf coast sturgeon, Common sturgeon and Sea sturgeon (Barkuloo, 1988, Huff 1975, US Fish and Wildlife Service, 1995) For a long time, the species *Acipenser oxyrinchus* was considered to be a subspecies of the European sea sturgeon (*Acipenser sturio*), owning to their morphological similarities (Huff, 1975, Birstein, 1993) In the beginning of the 1800s it was recognized that the American Atlantic sturgeon belonged to a different species (Birstein 1993)

Nevertheless, it was just in 1955 that Vladykov conducted a study showing that even though Gulf sturgeon belonged to the species *oxyrinchus*, it was distinctly different than Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) (Vladykov 1955). Therefore, a new southern subspecies was assigned in honor to Fernando de Soto, a Spanish captain from the 16th century who explored the Gulf of Mexico region (Vladykov, 1955) The main differences found between the two subspecies are the following. 1) larger head length and slightly longer pectoral fins on Gulf sturgeon, 2) shape of the scoutes, 3) length of the spleen, which is 16-19% of fork length in Gulf sturgeon and 3-9% in Atlantic (Wooley and Crateau, 1985) However, it seems that the only difference that unequivocally distinguishes the two subspecies is the length of the spleen (Bowen and Avise, 1990)

One of the best general descriptions of the Gulf sturgeon was found in the Gulf Sturgeon Recovery Plan depicts the fish as

> Gulf sturgeons are anadromous fish with a sub-cylindrical body imbedded with bony plates or scoutes The snout is greatly extended and bladelike with four fleshy barbels in front of the mouth which is protractila on the

lower surface of the head. The upper lobe of the tail is longer than the lower lobe [Figure 2-1]. The subspecies is light brown to dark brown in color and pale underneath (US Fish and Wildlife Service, 1995, pp.2)

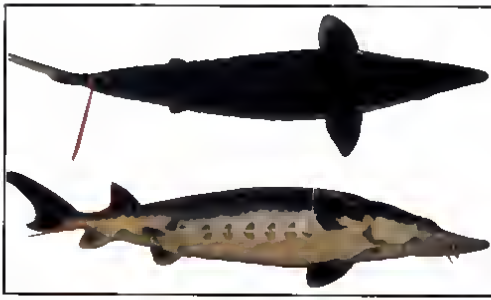

Figure 2-1 *Acipenser oxyrinchus desotoi*

Biological Characteristics

Several researches consider Gulf sturgeon an anadromous fish. Nonetheless, adult and sub adult Gulf sturgeons spend about eight to nine month of the year in the rivers, and just three or four of the coolest months in estuarine of Gulf waters (US Fish and Wildlife Service, 1995). In addition, it seems that fish less than two years old spend the whole year in riverine and estuarine areas (US Fish and Wildlife Service, 1995). According to Doroshov's definition of migration patterns for sturgeon species, this particular subspecies should be actually considered semi anadromous.

The movements of Gulf sturgeon have been monitored in several rivers such as Apalachicola, Suwannee, Pearl and Choctawhatchee (US Fish and Wildlife Service,

1995) Normally this is accomplished by ultrasonic and radio telemetry and by conventional fish sampling (US Fish and Wildlife Service, 1995). In general, upstream migration of adults and sub adults in Florida occurs from February through April, while the return to estuarine and marine waters starts in late October or November (Barkuloo, 1988) It is interesting to note that Gulf sturgeons move along the river seasonally For example, sturgeons in the Suwannee River spend summer months near the mouths of springs and cool water rivers (US Fish and Wildlife Service, 1995). Very little is known about the marine habitats of Gulf sturgeons, but there is some evidence indicating that sand bottoms, hard bottoms and sea grass communities are very important for Gulf of Mexico sturgeon

Studies on radio-tagged fish indicate that sturgeons are genetically or behaviorally imprinted to the chemosensory environment of their home rivers (Wooley and Crateau, 1985). In addition, restriction fragment length polymorphism (RFLP) analysis and sequence analyses of mitochondrial DNA of *A oxyrhinchus desotoi* indicate extensive structuring of Gulf sturgeon populations (Stabile et al., 1996) Apparently, there are at least four genetically distinct stocks in drainages along the Gulf of Mexico coast (Stabile et al., 1996) Gene flow estimates for the different stocks are low, indicating strong maternal home fidelity (Stabile et al 1996) Homing fidelity of Gulf sturgeon is considered to be high, not only on a region-specific basis, but also on a river-specific basis (Stabile et al., 1996)

Wooley and Crateau (1985) noticed the peculiar feeding habits of Gulf sturgeon Based on study results of the Suwannee and Apalachicola River sturgeons, *A oxyrhinchus desotoi* feed in fresh and brackish water until about age three (Barkuloo, 1988). After

age five they are believed to feed almost entirely in marine or estuarine waters during winter months and live off stored body fat while in the rivers Large sturgeons can lose up to 17% of their body weight during summer while in fresh water and gain up to 136% in marine and estuarine waters (Barkuloo 1988) In a most comprehensive study about feeding habits of Gulf sturgeon in the Suwannee River, Mason et al (1993) also verify that sub-adults of more than 5 kg and adults in freshwater environment essentially fasted during summer and fall It seems that the fish is indifferent to the abundant potential freshwater foods and apparently had stored sufficient nutrient reserves while in the estuary (Mason et al , 1993)

The first study about age and growth of Gulf sturgeon from the Suwannee River was performed by Huff (1975) The study consisted of 301 males and 331 females ranging in fork length from 30-188 cm (Huff 1975) Pectoral fin rays were used to estimate age and mean fork lengths for fish age 1 through 17 (sexes combined) were determined (Huff, 1975) In addition, length-weight relationships were calculated and nine regression lines were developed according to sex, season and method of capture (Huff, 1975) Cross sections of pectoral fin rays were also used to estimate the age of 76 sturgeons collected from the Apalachicola River from 1982 to 1990 (US Fish and Wildlife Service, 1995).

Initial attempts to collect Gulf sturgeon eggs and larvae were unsuccessful Wooley et al (1982) and later on Foster et al (1988) were the first workers to report collection of wild larvae (US Fish and Wildlife Service, 1995) In addition, just a few unpublished manuscripts report collection of Gulf sturgeon eggs (US Fish and Wildlife Service, 1995) Therefore, timing, location and habitat requirements for Gulf of Mexico

sturgeon spawning remains unclear (US Fish and Wildlife Service, 1995) Apparently, the eggs are demersal, adhesive, globular, and coloration varies from gray to brown to black (US Fish and Wildlife Service, 1995) Parauka et al. (1991) reported that the eggs averaged 2 10-2 20 mm in diameter Finally, it can be mentioned that *A. o. desotoi* reaches sexual maturity rather late Mature females range in age from 8 to 17 years while males range form 7 to 21 years (Huff, 1975)

Geographical Distribution and Populations

Gulf sturgeons are known to occur in the northern Gulf of Mexico, in most major rivers from the Mississippi River to the Suwannee River (Wooley and Crateau, 1985) Historically, sturgeons were also found in the Tampa Bay area. They would even move southward along the western Florida coast all the way to Florida Bay in due to exceptionally cool weather (Wooley and Crateau, 1985) The historic range of Gulf sturgeon is depicted in Figure 2-2 (US Fish and Wildlife Service, 1995) Unfortunately, overexploitation and habitat destruction greatly contributed to the disappearance of Gulf sturgeons from their historic range (Wooley and Crateau, 1985) Since 1890, only occasional catches have been reported in the Tampa Bay area, and this population is considered extinct (Barkuloo 1988) Population of Bear Creek and Bay County shared the same fate A dam was placed across North Bay downstream from Bear Creek in 1962 preventing anadromous fish migration (Barkuloo, 1988) Nowadays, population estimates are unknown throughout its range Occurrences of Gulf sturgeon are basically founded on scattered reports of incidental catches The only population estimates that have been completed are for the Apalachicola and Suwannee rivers (US Fish and Wildlife Service, 1995)

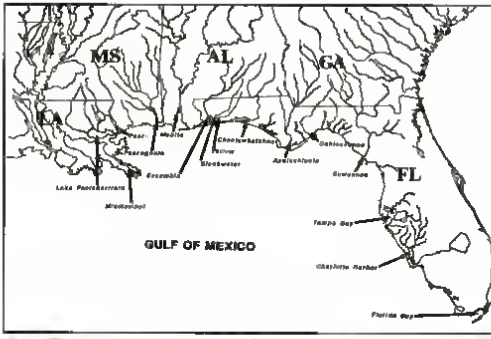

Figure 2.2 Historic range of Gulf of Mexico sturgeon Source US Fish and Wildlife Service, 1995

Gulf sturgeon population has been monitored the Apalachicola River since 1979 Approximately 350 Gulf sturgeon were collected, tagged and recaptured below Jim Woodruff Lock and Dam (JWLD), from 1981 through 1993 (US Fish and Wildlife Service 1995) Since 1984, the estimated annual number of fish ranged from 96 to 131 with a mean of 115 units It is important to mention that in this study, fish smaller than 45 cm in fork length were excluded because of sampling bias caused by net selectivity (US Fish and Wildlife Service 1995)

The Suwannee River probably has the healthiest and most viable population of *A oxyhrinchus desotoi* in the Gulf of Mexico coastal rivers (Barkuloo, 1988). The Caribbean Conservation Corporation (CCC) has captured, marked and released 1,670 sprug migration fish at the river mouth since 1986 (US Fish and Wildlife Service, 1995)

The annual estimated population size ranged from 2,250 to 3,300 fish averaging about 18 kg (39 7 LB) (US Fish and Wildlife Service, 1995) The Suwannee River maintained the most consistent commercial fishery for Gulf sturgeon since 1970, and until 1984 when the State of Florida prohibited harvest and possession (Barkuloo, 1988, US Fish and Wildlife Service, 1995) Adult population of sturgeon in the river was drastically reduced following large commercial catches in 1983 and 1984 (Barkuloo, 1988)

A Threatened Subspecies

> The US Fish and Wildlife Service (FWS) and National Marine Fisheries Service (NMFS) designated the Gulf sturgeon to be a threatened subspecies pursuant to the Endangered Species Act of 1973, as amended (ESA) The listing became official on September 30 1991 As part of the listing, a special rule was promulgated to allow taking of subspecies for educational purposes scientific purposes the enhancement of propagation or survival of the subspecies zoological exhibition and other conservation purposes consistent with the ESA (US Fish and Wildlife Service, 1995 pp 2)

Records show that the first sturgeon fishery started in Tampa Bay in 1886 (Huff, 1975) The fishery was conducted only in winters and it remained in operation for a very short period of time (Huff, 1975). In 1890 the stock in the area was already depleted with just seven fish caught in the previous season, the fishery was then abandoned (Huff 1975) A few years later a gill-net fishery was installed in the Suwannee River, and its primary business was the sale of flesh rather than caviar (Huff, 1975) In the beginning of the 1900's, several fisheries in the northwest Florida were commercializing meat and caviar (Huff, 1975) In 1917 it was reported that drift nets with six-inch mesh were being used and that the fish size was rapidly declining (Huff, 1975) Sturgeon landings and catch statistic reports can be found for some regions in specific years However, there are several gaps throughout the years and some of the data is also incomplete (Huff, 1975)

Aquaculture

Aquaculture of any sturgeon species is promising. Eggs can be marketed at high prices, and flesh is considered as premium-priced meat. Smoked sturgeon is a value added product marketed in many regions in the world (Bardi, 1997). In the USA, artificial propagation of white sturgeon (*Acipenser transmontanus*) gave rise to a profitable commercial aquaculture industry in California (Bardi 1997). Hormone-induced ovulation and spawning of Gulf of Mexico sturgeon took place for the first time in 1989 at a hatchery located on the Suwannee River (Welaka National Fish Hatchery, FL) (US Fish and Wildlife Service, 1995). During subsequent years, the University of Florida in conjunction with the FWS and CCC produced about 60,000 fry for fish culture programs (US Fish and Wildlife Service, 1995). In the event aquaculture of Gulf sturgeon becomes approved in the state of Florida, the University of Florida Demonstration Farm facility located in Blountstown (FL) will be able to provide technical support to local farmers. Like other sturgeon species, there is great potential for commercialization of Gulf of Mexico Sturgeon raw and smoked white muscle as well as caviar, in the domestic and international market.

Smoking of Sturgeon Fillets

Traditionally sturgeon meat is smoked, but the literature regarding smoking of sturgeon meat is scare. Smoking and drying are ancient processes primarily used to preserve or extend the shelf-life of food products (Bligh et al., 1988). Smoked and dried fish are still widely consumed in many developing countries as a major source of inexpensive dietary protein (Poulter, 1988). Nevertheless, techniques such as refrigeration, cold storage and thermal processing have been substituting older

preservation methods in the food industries all over the world Presently smoking can be viewed as a stage of processing intended to enhance texture and flavor of a food product (Poulter, 1988)

Methods of drying and smoking of fish can vary extensively depending on the species of fish, the type of product desired as well as the traditions of a specific country (Opstvedt 1988) The process may use unsalted fish or produce a variety of salt concentration in the final product, ranging from less than 2% and up to 20%. Curing agents as nitrates may be added (Opstvedt, 1988) Dehydration may be applied and degrees of moisture can vary from 10% to 60%. The fish maybe dried, smoked or a combination of both (Opstvedt, 1988) Generally, smoked fish can be placed into two categories cold and hot Cold smoking is carried out at temperatures ranging from 15^{0}C to 30^{0}C (average 25^{0}C), while hot smoking can be further divided into mild or high temperature process (Motohiro, 1988) Mild temperature smoking uses temperatures between 30^{0}C to 50^{0}C, and high temperature process is carried out at temperatures ranging from 50^{0}C up to 80^{0}C (Motohiro, 1988) The requirements for salt content are lower for hot smoking than for cold smoking. The time required to hot smoke a product (normally 3 to 8 h) is much shorter than to cold smoke it (Motohiro, 1988)

Smoking of sturgeon meat can be considered a value added process (Chapman et al , 1997) A study carried out with White sturgeon fillets (*Acipenser transmontanus*), using sodium potassium phosphate (Carnal 2110) Added to the brining solution prior to smoking, showed that it significantly improved color development. The fish were darker and more evenly colored after phosphate treatment. Carnal 2110 also increased water

retention during smoking, thus improving processing yields. Besides it was shown to causes no detrimental effects in consumer acceptability (Chapman et al., 1997)

The use of phosphates and polyphosphates in fish fillets is well described in the literature (Regenstein and Pedrosa-Menabrito, 1988), and it can be pointed out that polyphosphates interact with food constituents performing several functions

- Inactivation of metal ions by precipitating the ion out of solution or by formation of a complex,
- Maintenance of optimum pH values (buffering effect),
- Increase of hydration and water binding through interaction of proteins, leading to higher retention of natural juices and lower loss of soluble constituents.
- Preservation by inhibiting growth of some microorganisms and retarding oxidation of unsaturated fatty acids.

In addition, phosphates can inhibit dehydration of the muscle tissue by solubilizing certain proteins on the surface of fish fillet. The protein forms a layer of gel on the surface that basically seals it, preventing loss of fluids. This will slow down oxygen diffusion into the tissue, thus retarding oxidation of fats (Regenstein and Pedrosa-Menabrito, 1988)

Rigor Mortis

During harvest, aquatic animals experience high activity levels. The metabolism speeds up, oxygen demand increases and the animal struggles trying to escape and survive (Wheaton and Lawson, 1985). Harvest stress can greatly affect quality. Bruising, physical injury and removal of slime coat can be detrimental to the food product

(Wheaton and Lawson, 1985). In addition, the handling conditions post mortem will greatly affect the quality of the product due to rigor mortis.

Rigor Mortis (Latin 'the stiffness of death') is one of the most drastic changes that occur in the muscle soon after death (Gill et al., 1998). Rigor is characterized by extended muscles contractions. Thus, it is important to have some understanding of the structure of fish muscle and the biochemical events postmortem, in order to value the importance of rigor mortis on the quality of fish muscle.

Fish Muscle Structure

Muscle function does not differ between terrestrial and aquatic animal. It basically provides the necessary structure for locomotion (Hultin, 1995). However, there are several differences in muscle composition and structure between animals, species and even within the same animal depending on the specific function of a muscle (Hultin, 1985). Fish muscle presents some unique characteristics, when compared to terrestrial animals. Fish body is supported by water and it does not require extensive, strong connective tissue (Hultin, 1985). The structural arrangement of the muscle is closely related to the way fish flex their bodies for propulsion in water (Eskin et al., 1971; Hultin, 1985). In addition, since most important commercial species come from cold water, proteins in fish muscle have markedly different properties than those of warm-blooded animals (Hultin, 1985).

Muscle can be classified into three types: striated (voluntary) muscle that constitutes the lean meat, unstriaded (involuntary) muscle found in the stomach walls, and cardiac muscle (Eskin et al., 1971). Fiber is the basic unit of a muscle. It is a cylindrical cell with several nuclei bounded by an outer membrane denominated sarcolemma (Eskin et al., 1971). Its diameter ranges from 10 to 100 micrometers while

length can vary from a few to several centimeters long (Huhn, 1985) Fiber bundles associate groups of fibers, and a single muscle is formed by several budles held together by connective tissue, also called perimysium (Eskin et al , 1971) Perimysium is covered by another type of connective tissue called epimysium (Eskin et al., 1971) Fibers arranged between sheets of connective tissue are much shorter in fish than in warm-blooded animals (Eskin et al., 1971)

Fish muscle is composed of myotomes, W-shaped segments showing one forward flexure and two backward flexures (Lagler et al 1977) Myotomes are one cell deep intercepting the vertical mid plane of the fish body at sharp angles (Figure 2-3 and Figure 2 4) (Lagler et al , 1977) Muscle cells are arranged perpendicularly to the surface of the myotome, and myotomes are connected together by a collagenous tissue named myoseptu or myoconnneta (Lagler et al., 1977) The myotome patterns of Gulf sturgeon can be seen in Figure 2-5

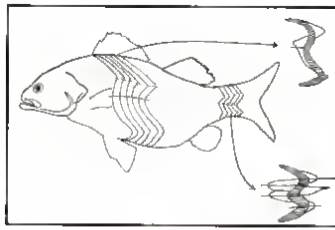

Figure 2-3 Lateral view of myotome pattern in bony fish muscle
Source Lagler et al 1977

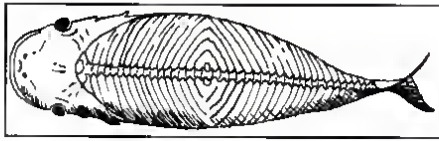

Figure 2-4 Top view of myotome pattern in bony fish muscle Source Laggler et al , 1977

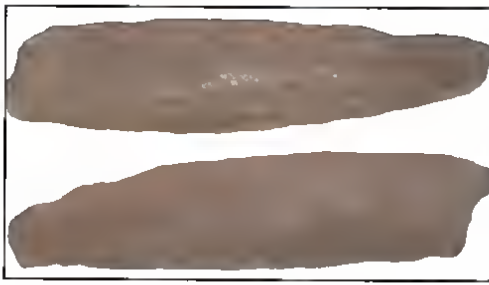

Figure 2-5 Myotome pattern in Gulf sturgeon muscle

The major components of a typical muscle fiber are sarcolemma, myofibrils, sarcoplasm, mitochondria and sarcoplasmic reticulum (Eskin et al , 1971; Hultin, 1985) The surface of the muscle is the sarcolemma (Eskin et al., 1971) It consists of three layers an outer layer of collagen fibrils, a middle amorphous layer, and an inner layer of plasma membrane (Hultin, 1985) The plasma membrane is extended to the interior of the muscle cell allowing the cell to respond as a unit. There is basically no lag period

between the external areas and the interior of the cell during contraction (Hultin, 1985) Myofibrils are the major inner components of the muscle fiber and are responsible for anatomical features of the muscle (Eskin, 1971). Myofibrils are cross-striated and composed of two main types of filaments. The thick filaments are mainly composed of myosin and the thin filaments basically composed of actin (Eskin, 1971) Myofibrils are separated from each other by a fine network of tubules, known as sarcoplasmic reticulum (Eskin et al , 1971) Sarcoplasm is the cell fluid, or the liquid matrix within the fiber; it is composed of several proteins, enzymes myoglobin, glycogen, ATP and creatine (Eskin et al , 1971) Mitochondria are organelles located throughout the cells and they serve as the main energy transducers (Hultin, 1985).

Biochemical Events Postmortem

Muscle works by converting chemical energy into mechanical energy Consequently, it requires a high energy level to operate the contractile apparatus (Hultin, 1985). This energy is obtained from ATP (adenosine triphosphate) Atkinson and Walton (1967) defined the energy level of a cell as "energy charge' This is a function of ATP concentration relative to the concentration of its breakdown products ADP (adenosine diphosphate) and AMP (adenosine monophosphate), as shown below

$$\text{Energy charge} = (ATP + \tfrac{1}{2} ADP) / (ATP + ADP + AMP)$$

In living muscle, ATP is normally obtained from respiring mitochondria that utilize molecular oxygen (Hultin 1984) When circulation stops, oxygen is no longer available thus ATP cannot be produced by aerobic respiration. Nonetheless, muscle cells will keep trying to maintain a high energy level through anaerobic pathways Generally the ATP content of muscles is about 3-5 mg/g of fresh muscle at the moment

of slaughter. Whenever ATP concentration reaches about 1 μmol/g, onset of rigor is observed (Gill et al., 1998).

ATP can be generated by an enzymatic reaction (creatine kinase) where creatine phosphate donates its phosphate group to an ADP molecule (Watabe et al., 1989a). Another ready available enzymatic pathway (adenylate kinase) to produce ATP is the conversion of two molecules of ADP into one molecule of ATP and one molecule of AMP (Hultin, 1984). However, these are limited processes because the resources are generally depleted within a short period postmortem (Hultin, 1984). Glycolysis is normally the major anaerobic source of ATP production after death. Glycolytic enzymes can convert glycogen or glucose to lactate and ATP through a series of phosphorylated sugar derivatives (Hultin, 1984). Nonetheless, anaerobic glycolysis yields only 2 to 3 moles of ATP per mol of substrate while aerobic respiration yields about 36 to 37 moles of ATP. Moreover, the only by products of aerobic respiration are water and carbon dioxide, while anaerobic glycolysis results in an incomplete oxidation of substrates, leading to lactate accumulation.

When ATP is broken down through hydrolysis, hydrogen ion are produced lowering the pH of the tissue. Notice that the pH initially drops slowly, generally followed by an increase, but it normally will never return to its pre-rigor level (Wheaton and Lawson, 1985). Drop in pH causes inhibition of glycolytic enzymes, such as phosphofructokinase (Hultin, 1985). Accordingly, ATP production ceases. The pH at which this happens is normally around 5.1-5.5 (Hultin, 1985). Another possible mechanism to disrupt ATP production is depletion of the glycogen reservoir, but this is not common in fish muscles (Izquierdo-Pulido et al., 1992). An increase in enzymatic

activity in muscle cells can also speed up ATP breakdown (Hultin, 1985). This is mainly due to loss of calcium-sequestering ability by the sarcoplasmic reticulum and mitochondria in muscle cells (Hultin, 1985). The disruption of the energy-generating systems combined with the development of anoxic conditions after death cause an increase in permeability in the membrane of these organelles (Hultin, 1985). Therefore, calcium ions leak into the sarcoplasmic reticulum interacting with contractile proteins. This interaction increases ATPase activity in membrane systems. A study of myofibrillar Mg^{+2}ATPase activity and calcium uptake by sarcoplasmic reticulum showed that progress of rigor correlates well with Mg^{+2}ATPase activity in the presence of a few micromoles of calcium ions at 0^{0}C (Watanabe et al., 1989b).

An important aspect of ATP degradation is the formation of flavor enhancers as intermediates in the production of hypoxanthine (Figure 2-6) (Hultin, 1985). Nucleotides having a phosphate group in position 5' or a hydroxyl group at position 6 will enhance flavors in muscle foods (Hultin, 1985). Some examples are ribose 1-phosphate, inosine monophosphate (IMP), guanilic acid (GMP) and purine (Hultin, 1985). Notice that IMP eventually degrades to hypoxanthine, which imparts bitterness. It is believed that this is one possible cause of development of off flavors in stored fish (Hultin, 1985).

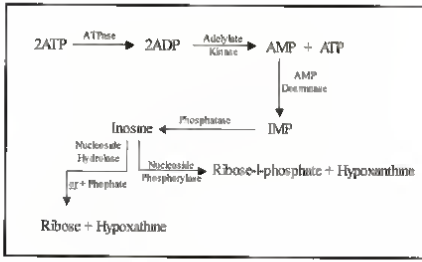

Figure 2-6 Convertion of ATP to hypoxanthine in postmortem muscle. Adapted from. Hultin, 1985

The biochemical cascade of events behind muscle contraction is not fully understood It is known that muscle contraction takes place by depolarization of plasma membrane by electrical nervous impulse (Hultin, 1985) This triggers the liberation of calcium ions from the sarcoplasmic reticulum (Hultin, 1985) The calcium ions activate ATPase on the contractile proteins by overcoming the inhibition of magnesium ions. (Hultin, 1985) Figure 2 7 depicts the sliding filament model of muscle contraction (Hickman et al., 1986) These bridges swing back and forward in an alternate pattern to draw the thin filament past the thin filamcot in a ratchet action (Hickman et al , 1986). The thick and thin filaments, myosin and actin respectively, become linked together by crossbridges that act like levers pulling the filaments pass each other (Hickman et al , 1986) This 'attach-pull-release' mechanism occurs in cycles through a series of steps as shown in Figure 2-8 Notice that each cycle requires the expenditure of 1 ATP molecule (Hickman et al , 1986)

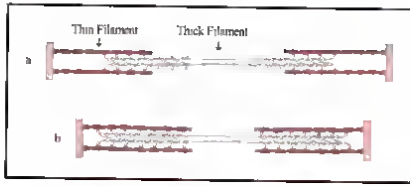

Figure 2-7 Sliding filament model (a) Muscle relaxed, (b) Muscle contracted. Adapted from Hickman et al., 1986

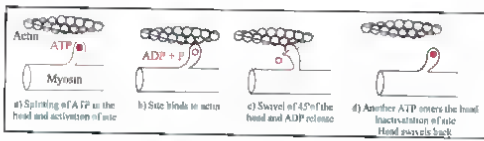
Actin
ATP
Myosin
a) Splitting of ATP as the head and activation of site
ADP + P
b) Site binds to actin
c) Swivel of 45° of the head and ADP release
d) Another ATP enters the head Inactivation of site Head pushes back

Figure 2-8 Attach-pull-release cycle of sliding filament model. Adapted from Hickman et al., 1986

Drop in ATP levels leads to impediment of crossbridge cycling between myosin and actin in the myofibrils (Hultin, 1984). ATP can be considered a plasticizer of these filaments because it prevents permanent linkage. However, when concentration of ATP lowered down, muscle looses its natural extensibility (Hultin, 1984). The permanent formation of actomyosin after ATP is depleted continued to the extent of overlapping between thin and thick filaments will greatly influence toughness in post rigor fish fillets (Hultin 1985).

Variables Affecting Rigor Mortis

The timing of onset and resolution of rigor mortis can be very different depending on the species. The patterns of ATP breakdown postmortem are dependent on the species (Gill, 1990) The speed of nucleotide degradation is influenced by killing method and amount of struggling prior to death, delay in bleeding the fish, sexual maturity, feeding habits, overall health of the animal poor handling technique and temperature during storage (Chiba et al , 1991, Love, 1988, Huss, 1988). It is noteworthy to mention that, contrary to other animals, the ultimate pH of fish is not related to stress prior to death (Hultin, 1985)

Salmon killed by a blow to the head enter rigor about eighteen hours after death, but if stunned by carbon dioxide and killed by bleeding, it becomes rigid in five hours (Love, 1988) A study comparing rigor progression of red sea bream in relation to killing method and season shows that both factors play an important role in textural quality of the product (Nakayama et al., 1999). Handling stress leads to shorter pre-rigor period in farmed Atlantic salmon, influencing textural quality of the muscle (Sigholt et al., 1997) Similar results were found in wild Atlantic salmon in an experiment run at a commercial fish processing plant in central Norway (Berg et al , 1997) Studies conducted on White sturgeon muscle show that fish allowed to struggle prior to death have much faster ATP depletion than anesthetized fish (Izquierdo-Pulido et al , 1992) Penetrometer tests revealed that White sturgeon cooked fillets had firmer texture pre rigor than post rigor In addition, post rigor fillets from struggled fish were softer than the ones from anesthetized fish (Izquierdo-Pulido et al., 1992)

Fish that are filleted before resolution of rigor can shrink up to half of its former length when cooked (Love, 1988 Huss 1988) However, if the fillet remains attached to the skeleton, which prevents shortening, then little change in texture is noticed after cooking (Lova, 1988) Actually this might not be true if rigor develops at high temperatures (in case of cod this is above 17°C) As the rigor tension may become too strong causing weakening of the connective tissue and rupture of the fillet, a phenomenon known as 'gapping' (Huss, 1988)

Starving fish can enter rigor almost immediately after death, and remain in rigor for very short period of time This is probably due to the fact that starving fish have a small glycogen reservoir (Love, 1988)

Temperature is also critical to the onset of rigor mortis, which occurs more rapidly if fish are not chilled quickly after death (Iwamoto et al , 1987, Watabe et al., 1989a) However there is some controversy on the relationship between rigor-mortis progress and temperature (Watabe et al , 1989a, Huss, 1988) It was reported that several fish species such as sardine, mackerel and plaice had their pre-rigor periods shortened when stored at 0°C compared to warmer temperatures (Iwamoto et al., 1987, Watabe et al., 1989a). While some other species such as cod, mahi mahi and grunt seem to have the onset of rigor retarded at 0°C (Watabe et al , 1989a) It is known that bacterial spoilage normally does not take place before complete resolution of rigor, it is still important to keep the catch at low temperatures to maintain the bacteriological quality of the product

Recently it was determined that rigor can influence the salt uptake and diffusion in Atlantic salmon muscle during brining (Gill et al., 1998, Wang et al., 2000). The equilibrium salt concentrations of pre-rigor, in-rigor and post-rigor mortis salmon were

0 53 0.66 and 0 75 g/g salt-free solids respectively, in a 20% (w/v) sodium chloride solution at 100°C (Gill et al , 1998) Moreover, salt distribution inside pre-rigor salmon samples was less uniform than in-rigor and post-rigor (Wang et al., 2000)

There is evidence showing that onset of rigor in White sturgeon (*Acipenser transmontanus*) ranges from 72 h to more than 96 h (Izquierdo-Pulido et al., 1992) In addition White sturgeon can take as long as seven days to resolve rigor (Izquierdo-Pulido et al., 1992) Accordingly, it is important to monitor rigor mortis in Gulf sturgeon to determine its time of onset, duration and resolution, in order to ensure optimal quality of the meat by the time it reaches the consumer

Chemical Methods of Monitoring Rigor

There are a plethora of methods described in the literature to monitor chemical compounds involved in postmortem biochemistry of fish muscle. A review of these methods is beyond the scope of the present work It is noteworthy to mention that concentrations of ATP, ADP, AMP, IMP, inosine, and hypoxanthine can be measured by HPLC Surette and his coworkers (1988) thoroughly examined the enzymatic changes involved in postmortem breakdown of nucleosides in cod Generally, a portion of the muscle is added to a perchloric solution, and the procedure that follows is variable in accordance to intrinsic characteristics of the sample and equipment Concentration of lactate can be enzymatically determined in perchloric acid extracts of fish muscle (Watabe et al. 1991a) Creatine phosphate concentrations are also determined by HPLC (Watabe et al., 1991a) Myofibrillar ATPase assays are normally performed to measure its activity (Watabe et al , 1989b, Watabe et al , 1991b, Wang et al , 1999) Finally calcium ions can be quantified by liquid scintillation counter using Aquasol II coktail as

scintillant (Watabe et al., 1989b Watabe et al , 1991b), by calcium sensitive electrode or by titration.

Physical Methods of Monitoring Rigor

During the course of the present work a novel method of monitoring progression of rigor mortis in fish body was developed. Therefore, it is pertinent to present a brief review of the physical methods of monitoring rigor found in the literature

Instron

Breaking strength of fish fillets can be used to follow rigor progression The fillets are stretched at 1 cm/min and the necessary force to break the fillet is recorded (Sigholt et al 1997) Force of compression is another variable used to measure rigor progression An aluminum plunger is used either perpendicular to or parallel to the long axis of the fibers (Izquierdo-Pulido et al , 1992) Compression force applied parallel to the fibers is an estimate of cohesiveness, while force applied perpendicularly is an estimation of firmness (Izquierdo Pulido et al , 1992)

Rigor index

Bito and collaborators developed this index in 1983 (Iwamoto et al , 1987) This is the most accepted physical measurement to monitor rigor mortis progression over time Assessment of rigor state using this method in conjunction with other techniques such as sensory evaluation, texture measurements pH progression and quantification of specific biochemical changes is often found in the literature (Iwamoto et al 1987 Izquierdo Pulido et al , 1992, Watabe et al., 1989a, Watabe et al 1991a, Toyahara and Shimizu, 1988 Gill et al., 1988, Wang et al., 2000) It can be seen in Figure 2-9 that the procedure is very simple Half the length of the fish body is placed on a horizontal table while the tail part sags down (Iwamoto et al , 1987) The initial distance between the table surface

and the tail is recorded. At selected time intervals the measurement is recorded again, and Rigor index calculated according to the equation below (Iwamoto et al., 1987)

$$\text{Rigor Index } (\%) = [(Do - D) / D] \times 100$$

Where
Do = distance from table to tail at time zero
D = distance from table to tail at any given interval of time

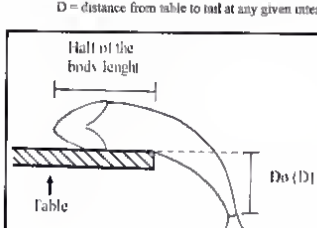

Figure 2-9 Apparatus to determine Rigor Index. Source: Iwamoto et al., 1987

Low frequency vibration instrument

This instrument was used to access the effects of handling stress prior to slaughter in rigor mortis progress of Atlantic salmon. The equipment shown on Figure 2-10 is essentially based on an electrical signal source, a vibrator and two sensors (Berg et al., 1997). Atlantic salmon was placed on a thin plastic film touching the upper part of the vibrator, while a wooden frame holds the plastic film. The fish is exposed to a range of low frequency waves for 3 to 5 seconds. A portable computer controls sensors and source and all measured data is logged on file (Berg et al., 1997). A multilayered feed forward neural network was used to analyze the data. The authors concluded that

performance of the equipment was not as high as expected due to the complexity of the data and large variation from fish to fish within the same groups (Berg et al , 1997) It was suggested that a higher number of experimental units have to be tested and the positioning of the vibrator optimized Nonetheless, it is a gentle, nondestructive technique that can be automated to fit on a slaughter line (Berg et al., 1997)

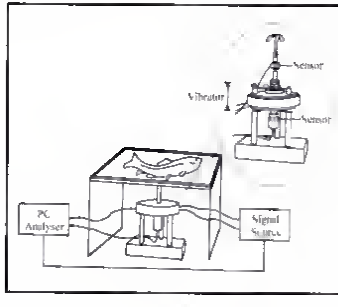

Figure 2-10 Low frequency vibration instrument Source Berg et al, 1997

Sag angle

This method measures progression of stiffness by observing the sag of the head when fish is clamped in a vertical position by the tail, as depicted in Figure 2-11 (Kerhonen et al., 1990)

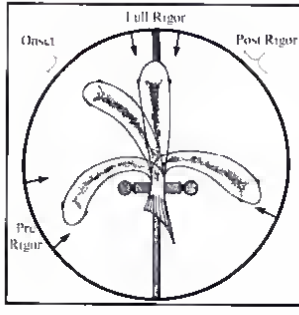

Figure 2-11 Apparatus to measure sag angle Source, Korhonen et al , 1990

Accordingly, the angle of sag divides the time postmortem into four stages pre-rigor, onset period, in-rigor period, and post rigor This technique was used to assess rigor in tilapia. This method appears to be mainly suitable for small size fish because it would be hard to place large size fish in this position Moreover, due to fish weight, large fish would never reach the position defined as full rigor

Rigorometer

Korhonen et al. (1990) constructed a tension rigorometer as shown in Figure 2-12 A 7 cm long stripe of tilapia white muscle, excised from the dorsal area is suspended in a beaker containing cold physiological saline solution The muscle is clamped at both ends, one clamp is attached to a weight at the base of the beaker while the other is attached to a nylon thread leading up to a fulcrum bar (Korhonen et al., 1990) The fulcrum of the bar is attached to a ring stand A second nylon thread is attached to the

opposite end of the bar, and leads downward to a small weight placed on an electronic balance (Korhonen et al., 1990). The balance is placed on a movable jack allowing the muscle stripe to be extended vertically by lowering the weighted end of the fulcrum bar. Initially the muscle stripe is vertical and with no apparent slack, and the scale is zeroed (Korhonen et al., 1990). At frequent intervals balance readings are recorded and then correlated with rigor progression. Results show a difference in pattern of rigor progression between stressed and unstressed fish. It was suggested that the method is suitable for distinguishing the relative physiological conditions of fish within a lot where the condition may vary by stress prior to death (Korhonen et al., 1990).

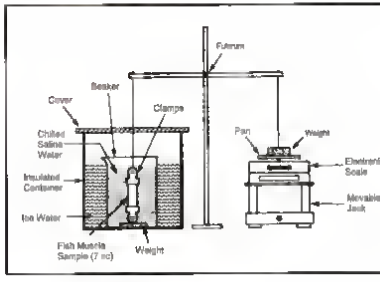

Figure 2-12 Rigorometer Source: Korhonen et al., 1990

Measurement of Contractile Percentage in Muscle

It was reported that the changes in muscle length of cultured red sea bream and Japanese flounder could be monitored during rigor using the apparatus depicted in Figure 2-13. The dorsal muscles, attached scales and skin were immediately removed after

killing (Lee et al , 1998) Approximately 1.5 x 1 5 x 10 cm of muscle was cut along the direction of the body from both sides of the fish body as shown in Figure 2-14 (Lee et al., 1998) The muscle stripe (scale side down) is placed on a glass plate in a constant temperature water bath (Lee et al , 1998) The contractile percentage of muscle is calculated as follows: [(A-B)/A] x 100, where A and B express muscle length at two consecutive measurements over time. The measurements were conducted at ten different temperatures ranging from 25°C to 3°C (Lee et al., 1998) Results show that cultured red sea bream presents faster temporal changes in muscle contraction than Japanese flounder at various storage temperatures between 25°C to 5°C In addition the peak level for red sea bream was remarkably higher In both fish species the progression speed of muscular contraction slowed with the decrease of storage temperature from 25°C to 12°C and became faster under 10°C (Lee et al., 1998). Jabarsyah et al (1999) determined muscle contractile percentage in chestnut puffer, threeline grunt, filefish and cultured red sea bream using this methodology Nakayama et al (1999), have also used this apparatus to evaluate the effects season and killing methods in rigor mortis of wild red sea bream on

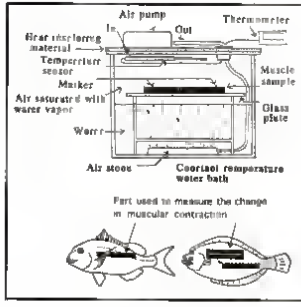

Figure 2-13 Apparatus to measure muscle contractions. Source Lee et al , 1998

Composition of Fish Muscle

The composition of fish muscle is affected by a number of environmental and metabolic factors Season, food availability, sexual maturity and spawning cycle, parasites or bacterial infection, stress, and water quality and temperature among others Muscle composition can drastically affect the nutritional value, flavor and aroma characteristics and texture properties of the final product (O'Keefe, 2000)

The most abundant compound of fish muscle is water, and it normally make take up to 85% of the total weight of a fish fillet. The proximate composition of several fish species found in the southern United States is presented in Table 2-2 It should be note that these are average values and seasonal variability in proximate composition can be significant

Table 2-2 Proximate composition of some fish species from Southeastern US

Species	% Moisture	% Protein	% Fat	% Ash
Channel catfish	82 1	16.2	2.4	1.0
Black sea bass	81 3	18.0	0.7	1.4
Gulf butterfish	77 5	20.0	3.9	1.2
Bluefish	77.5	20.0	2.4	1.1
Southern flounder	82.4	17.4	0.7	0.9
Yellowedge grouper	79.2	19.3	1.3	1.1
King mackerel	76.6	21 7	1.7	1.4
Striped mullet	74.4	21.0	1.7	1.4
Red porgy	77 1	22.0	0.9	1.5
Spotted sea trout	79.4	19.4	2.7	1.1
Red Snapper	77 8	20.4	1.4	1.2
Vermilhon Snapper	78.0	21 3	0.6	1.2

Adapted from Gooch et al , 1989

Proteins are the second most abundant compounds in fish muscle Proteins can be placed into three categories contractile, soluble and insoluble Soluble proteins are extracted with water or dilute salt solutions and contractile proteins are soluble in salt solution of high ionic strength (Hultin, 1985) Insoluble proteins are the remaining fraction after extraction of soluble and contractile proteins (Hultin, 1985) Fish muscle contains an average of 9 26% of crude protein, 70-80% of these proteins are structural while about 20-30% of fish protein are sarcoplasmic (Haard, 1995) Structural proteins include collagen (ca 10% of total protein) as well as myofibrils (contractile elements, 40 60% of total protein) (O'Keefe, 2000) The most abundant contractile proteins are myosin, actin actomyosin, tropomin and tropomyosin (O'Keefe 2000) The sarcoplasmic extract is composed of myoglobin, glycolytic enzymes, albumin and neoprotein constituents such as amino acids, lactate, nucleotides and carbohydrates (Hultin, 1985) The largest fraction of the insoluble components of a muscle cell is composed of connective proteins Some other insoluble compounds are unextractable contractile proteins such as connectin and desmin portions of the membrane system,

glycogen granules and fat droplets (Hultin, 1985). Collagen is the major fraction of connective tissue, and can also be found in tendons, skin, bone and the vascular system (Hulton, 1985). Fish muscle has lower amount of collagen, shorter and less connected muscle bundles than terrestrial animal because it does not require extensive support structure (O'Keefe, 2000). This results in a pleasant soft texture highly appreciated by consumers. Finally, fish protein contains all essential amino acids and it is highly digestible, thus fish muscle has excellent nutritive value (Haard, 1995).

Nonprotein nitrogen compounds (NPN) can be found in many species at levels up to 40%, however the range in lean fish is normally around 12%. These compounds can interfere with the quantification of protein in fish muscle, thus appropriate corrections on the results of Kjeldahl analysis are needed (O'Keefe, 2000). Free aminoacids, urea, trimethylamine (TMAO) and nucleotides are some examples of NPNs normally found in fish muscle.

Lipid content in fish and its influence in human nutrition has been the objective of several studies in the past years. The consumer is not adequately informed on seafood composition because of the variability in fat level in some species and problems in species nomenclature. In addition the origin of the fish is generally unknown to consumers (Ackman, 1990). Ackman (1990) has proposed a broad classification of seafood regarding its lipid content as follows:

- Lean (<2% fat) - cod, haddock, shellfish
- Low fat (2-4% fat) - sole, halibut, flounder
- Medium fat (4-8% fat) - salmon
- High fat (>8% fat) - herring, mackerel

Fat content is markedly different between dark and white muscle. Dark muscle contains higher levels of myoglobin, enzymes and fat than white muscle. This leads to rapid autolytic spoilage and loss of quality (O'Keefe, 2000). Whenever possible, removal of dark muscle is advisable during filleting to reduce lipid oxidation and consequently development of off flavors. Distribution and amounts of dark muscle are different among fish species as shown in Figure 2-14. Gulf sturgeon has a considerable amount of dark muscle. Under each row of bony plates that runs along the fish body there is a thick layer of dark muscle (Figure 2-15). Dark muscle can also be found between the skin and the white muscle as a thin sub dermal layer of fat. Black area around the fish body shown of Figure 2-15 corresponds to dark muscle distribution in Gulf sturgeon. Note that in order to decrease the rate of lipid oxidation, all dark muscle should be trimmed off the fillet's surface.

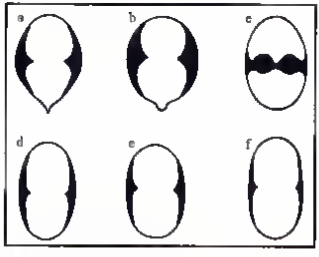

Figure 2-14 Sections through the bodies of several fish showing depth of dark muscle. (a) Herring, (b) Mackerel, (c) Tuna, (d) Haddock, (e) Cod, (f) Whiting. Source: Love, 1988

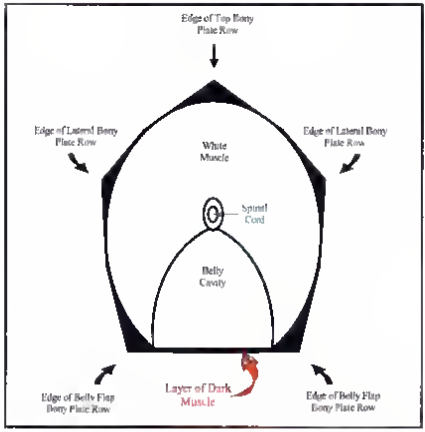
Edge of Top Bony Plate Row
Edge of Lateral Bony Plate Row
White Muscle
Edge of Lateral Bony Plate Row
Spinal Cord
Belly Cavity
Edge of Belly Flap Bony Plate Row
Layer of Dark Muscle
Edge of Belly Flap Bony Plate Row

Figure 2-15 Representation of dark muscle distribution in Gulf of Mexico sturgeon

The most common lipid classes found in fish muscle are triglycerides, phospholipids, sterols and wax esters (O'Keefe, 2000) Phospholipids are important constituents of cell membranes and are not used as energy reserve by the organism (O'Keefe, 2000) Triglycerides are the main source of energy in fish, and these are mainly stored in the liver of fish classified as lean Fatty fish have triglyceride reserves also in their muscle and belly flap area (O'Keefe, 2000) Fish lipids contain large amounts of long chain polyunsaturated fatty acids (Table2-3) (O'Keefe, 2000) The rate of oxidation is proportional to the number of unsaturated bonds in a fatty acid as well as

concentration of unsaturated fatty acids in relation to saturated ones (O'Keefe, 2000). It is important to point out that the fatty acid profile of marine fish species is quite different than freshwater species. Moreover, there are markedly differences in fatty acid profile of culture and wild fish of the same species (O'Keefe, 2000)

Table 2-3 Major fatty acids present in fish

Systematic Nomenclature	Common Name	Shorthand
Tetradecanoic acid	Myristic	14 0
Hexadecanoic acid	Palmitic	16 0
Octadecanoic acid	Stearic	18 0
cis-7-Hexadecanoic acid	Palmitoleic	16 1ω7
cis-9-Octadecanoic acid	Oleic	18 1ω9
cis-11-Eicosenoic acid	Gadoleic	20 1ω9
cis-11-Docosenoic acid	Cetoleic	22 1ω11
cis-9 cis-12-Hexadecadienoic acid	Linoleic	18 2ω6
all cis-9,12,15 Hexadecatrienoic acid	Linolenic	18 3ω3
all-cis 6,9,12,15 Hexadecatetranoic acid	Stearidonic	18 4ω3
all cis-5,8,11,14-Eicosatetraenoic acid	Arachidonic	20 2ω6
all-cis-5,8,11,14,17-Eicosapentenoic	Eicosapentenoic	20 5ω3
all-cis-7,10,13,16,19-Docosapentenoic acid	Docosapentenoic	22 5ω3
all-cis-4,7,10,13,16-Docosapentenoic acid		22 5ω6
all-cis-4,7,10,13,16,19 Docosahexenoic acid	Docosahexenoic	22 6ω3

Source: O'Keefe, 2000

Finally, it is noteworthy that consumption of omega 3 polyunsaturated fatty acids is beneficial to human health. Research in this area has shown that these compounds are essential in humans for proper growth and developmental processes, membrane structure and function, control and prevention of several diseases such as hypertension, diabetes, cardiovascular disorders, cancer, arthritis, psoriasis, and inflammatory and autoimmune conditions (Dyerberg, 1990)

Influence of Diet Composition on Muscle Quality of Aquaculture Species

Increasing knowledge of the health benefits of seafood consumption has led to increased demand for seafood products worldwide. Due to limitations of natural resources, several cultured fish species have been experiencing a growth in commercial importance. Under these circumstances, the need to investigate and determine the variables affecting quality of commercial products originating from aquaculture is evident.

During the last decade a great deal of research was conducted in order to determine the possible effects of diet composition on the muscle quality of cultured fish. In the early days, the best feed for cultured fish was chosen based solely on diet efficiency, which combines the results from best growth rate and lowest feed consumption (Nettleton, 1990). Presently, it is known that the choice of the most suitable feed has to include study of several quality variables of the fish muscle. Some of them are: proximate composition and fatty acid profile, sensory aspects such as color, flavor and texture of the meat and degree of lipid oxidation during storage. The nutritional value of aquaculture fish species as well as muscle quality will most likely be compromised by poor diet quality. As an example, it can be pointed out that results from a study on growth of Chinese sturgeon *Acipenser sinensis* show that fish fed a specific purified diet had higher moisture content, lower protein and lipid content when compared with fish fed live tubificid worms (Xiao et al., 1999). Nonetheless, the researchers were able to formulate a semi-moist practical diet that yields good rates of survival and similar muscle composition to fish fed live diet (Xiao et al., 1999).

Protein content seems to be similar between wild and cultured fish if all the essential amino acids are provided and well balanced, and feed has at least 30-35% of protein content (Lovell, 1991) On the other hand, fat content and fatty acid profile of cultured fish are greatly affected by the source of dietary lipids in the diet During the 80's the need to improve omega-3 content in cultured fish was pointed out (Piggot, 1989) These fatty acids are found in plants and animals associated with marine and fresh water environments (Pigott, 1989) At the time, it was determined that vegetable oils as only source of fat in the diet do not provided adequate nutrition for the fish It was suggested that fish oils should be added as source of omega-3 fatty acids (Piggot, 1989)

Because lipid composition in cultured fish closely reflects the composition of the dietary oil, it is possible to boost omega-3 levels in fish muscle (Nettleton 1990) Nonetheless, studies on farmed catfish showed that increasing levels of these fatty acids by providing larger amounts of menhaden oil in the diet led to development of undesirable fishy flavors, and higher rates of rancidity (Nettleton, 1990) In addition, studies on Atlantic salmon pointed out that sensory scores for fattiness, juiciness and taste intensity vary between fish fed different dietary levels of omega-3 fatty acids (Waagbo et al , 1993) Consequently, much care is needed in determine dietary lipid requirements for a specific fish species

Another interesting finding is that different species of fish have varying ability to desaturate and elongate fatty acids (Xu et al, 1993) Species with poor ability to elongate and desaturate fatty acids require higher amounts of highly unsaturated fatty acids than species showing high ability Cultured White sturgeon shows high ability to desaturate and elongate 18:2ω6 and 18 3ω3 thus they are able to utilize different lipid sources

equally well (Xu et al, 1993) However, diet formulated with cod liver oil was the only one to provided levels of omega-3 fatty acids in the muscle comparable to the ones found in wild White sturgeon (Xu et al., 1993)

Bardi (1997) investigated the survival and growth of larvae and juvenile Gulf sturgeon fed live and formulated diets The onset of feeding formulated diets led to nearly complete mortality Gulf sturgeon need to be fed live food for the first 4-5 weeks before acceptance of formulated diets, and this strategy is suitable to rearing fish for commercial aquaculture programs (Bardi, 1997) Gulf sturgeons were fed a semi-moist diet (Biodiet Starter-Bioproducts Inc) up to nine months of age, and then diet was switched to Silver Cup trout grower (Nelson & Sons Murray, Utah, USA, Bardi, 1997) Presently, commercial diets specially formulated for sturgeon are not available Gulf sturgeon reaches potential commercial size at 12 to 18 month of age Therefore, there is a need to investigate the possible influences of feeding different commercial diets on the quality of Gulf sturgeon muscle from fish reaching the estimated commercial size range Note that there were no studies on commercial size of Gulf sturgeon, and the estimations are based on information available on the literature for White sturgeon (Izquierdo-Pulido et al., 1992, Price et al , 1989)

Evaluation of Seafood Freshness Quality

St Angelo (1996) states that quality is a term that can be defined as the degree of excellence of a product Nevertheless, when applying this definition to food products it might be considered too broad or too simple from a scientific standpoint It can be pointed out that several approaches are currently used to define food quality, and that these are somehow related to the type or class of products under investigation (St Angelo

1996) The definition of quality presented by Molnar fits very well with the concept of St. Angelo, and it is as follows "Quality is a concept that is based on a number of product attributes that basically determine their level of suitability to a concrete and predetermined use." (Molnar, 1989, pp 1).

The definition of fish quality provided by Martin (1988) encompasses three main characteristics of the product and these are wholesomeness, integrity and freshness. Wholesomeness is defined as "a quality of a food fit to eat" uncontaminated and handled under good sanitary conditions. Integrity is defined as a product that well reflects the supplier's claims, and freshness is a quality of appearance, taste, odor and texture (Martin, 1988).

The handling of fresh fish is critical to quality assurance because fish are extremely perishable. Fish generally spoils faster than other muscle tissue, thus it should be handled with great care (Smith et al., 1996). The rate of deterioration is characteristic of individual fish as well as the mode of preservation. This fact is very much related to the variability of the muscle composition. However, in just the early part of this century this concept was acknowledged in a broader sense from the moment the fish is caught to the moment it is consumed. This was an attempt to minimize deterioration before the product reached the consumer, decreasing loses in the production and reducing the number of public health issues regarding seafood consumption (Smith et al. 1996). These current concerns are also a result of an effort of the seafood industry and affiliates in trying to maintain a recent trend of increase in seafood consumption in the US and several other countries (Smith et al., 1996).

Many consumers hesitate to include fish in their regular diets despite its health benefits. One of the main reasons consumers reject to consume fish is the presence of the so-called "fishy flavors" (Lindsay, 1990). However, it appears that fishiness is not a characteristic descriptor of freshly harvested fish and seafood, but normally present in less-than-fresh refrigerated and frozen fish and seafood (Lindsay, 1990). Accordingly, a great deal of research in the area of seafood quality and freshness has being carried out in order to enhance quality attributes and increase consumer acceptance.

There are several ways seafood quality can be assessed, the choice of a method will depend on the particular kind of seafood being evaluated, its intended use, sampling methodology, cost, time spend on analysis and destructibility of sample among others. Generally, the major practical methods involve chemical analysis, determination of physical parameters and evaluation of sensory attributes (Botta, 1995).

Major Chemical Methods

Storage leads to a variety of chemical changes in seafood and seafood products. Several of these changes have a great impact on many quality aspects, and must be controlled to ensure safety and suitability of the ultimate product to be marketed. The degree of microbiological spoilage, enzymatic activity and lipid oxidation can be monitored through quantitative analysis of some important chemical compounds formed during the process. The most important methods of analysis will be described.

Total volatile bases nitrogen (TVB-N)

Microbiological spoilage during postmortem storage causes the formation of volatile bases such as ammonia, dimethylamine (frozen storage mainly) and trimethylamine (Botta et al., 1984). The most important advantages of this method are speed, low cost and accuracy (Botta et al., 1984). As any method of analysis, this method

also has some drawbacks, one of the most important is the fact that TVB values are not linearly correlated to the length of time the product is stored (Perez-Villarreal and Howgate, 1987) Therefore storage life cannot be easily predicted On the other hand TVB values can be routinely used to identify if chilled, frozen, canned or dried seafood is spoiled Another important aspect is the fact that there are currently several different methodologies to carry out TVB value determinations (Botta, 1995) There is a considerable variation of TVB values between methodologies, thus it is important to clearly specify all of the steps involved in the procedure (Gill, 1990) If the detailed procedure is clearly followed, the results should be very accurate. TVB is normally used as an indication of advanced spoilage (Gill 1990)

Volatile acid number (VAN)

This method is very similar to TVB analysis, but the distillation of the extracts will provide volatile acids formed during spoilage (mainly formic and acetic acids), that can be further quantified and related to freshness (Tomiyama et al 1956) This is an old method seldom seen in the current literature as an evaluation of seafood decomposition.

Trimethylamine (TMA)

Marine fish species use TMAO (trimethylamine oxide) as an osmoregulator, thus these are highly susceptible to bacterial degradation of TMAO to TMA (trimethylamine), a volatile chemical with a fishy odor (O'Keefe 2000) It seems that most marine fish accumulate TMAO but levels are highest in gadoids and sharks (O'Keefe, 2000) Normally levels of TMA are low in earlier stages of spoilage, thus this index is most useful in medium-late stages of spoilage (Gill, 1990)

This chemical method has been extensively studied, and there are currently several methodologies available to measure TMA concentration in seafood (Botta, 1995)

Considering TMA values can be quite different depending on the methodology, it is very important to clearly specify the procedure (Botta, 1995) In addition, it appears that TMA levels can be affected by type of fish, season, location of catching type of processing (or storage), micro flora, stage of spoilage (Botta, 1995, Gill, 1990)

Ammonia

The major chemical compound in the volatile amines formed during process of spoilage is ammonia The usefulness of ammonia levels is mostly dependent upon the species being investigated (Halland and Njaa, 1955) It has been shown that ammonia levels are very useful index of freshness quality of shrimp, some crustaceans and cartilaginous fish (Botta, 1995). Some of the quantitative methods available to determine ammonia levels are diffusion accelerated microdiffusion, enzymatic conversion ion sensitive electrode and a test kit in the form of a paper strip (Botta, 1995)

Biogenec amines

Biogenic amines are formed by microbial decarboxylation of amino acids or other biogenetic amines. Agmantine, cadaverine, histamine, phenethylamine, tryptamine and putrescine are some examples of this class of compounds (Botta, 1995) The major problem of using this measurement as a freshness index is the fact that even though the presence of these amines is an indicator of spoilage, their absence is considered inconclusive to determination of the degree of spoilage in a product (Gill, 1990) The most common method is based on extraction of fish muscle derivatization into fluorescent or UV absorbing compounds and then HPLC analysis, thus it is expensive and time consuming (Yen and Hsish, 1991) Notice that consumption of fish containing high levels of biogenetic amines cause scombroid fish poisoning (O'Keefe, 2000)

Nucleotide degradation products

As it was mentioned erlier, nucleotide degradation starts to take place in fish muscle at death. Enzymes naturally present in the fish automatically start the breakdown of adenosine triphosphate (ATP) into several chemical compounds (Botta, 1995). The chemical products of this autolytic and microbial process are adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (Ino), hypoxanthine (Hx), xanthine (X) and uric acid (U) (Botta, 1995). The patterns of ATP breakdown postmortem are dependent on the species and temperature of storage (Gill 1990). It is important to emphasize that the speed of nucleotide degradation is influenced by the amount of struggling prior to death, delay in bleeding the fish, poor handling technique and temperature abuse during storage (Chiba et al., 1991). This method is considered the most suitable for very early stages of spoilage, as well as very useful index for freshwater fish (Botta, 1995). HPLC is normally used to measure the concentration of nucleotide degradation products. One of the major advantages over older procedures (basically wet chemical analysis) is that the concentration of all products can be measured simultaneously and it reflects both autolytic and microbial degradation (Surette et al., 1988). On the other hand HPLC is an expensive method of analysis and can be time consuming, which limits the usefulness for routine quality control (Botta, 1995).

The K value has been defined in order to provide a ratio that would relate nucleotide degradation to a decrease in freshness. It is the sum of Hx and Ino divided by the total of all ATP derived nucleotides as shown below (Saito et al., 1959)

$$K_1 \text{ value } (\%) = 100 * \{ ([Hx] + [Ino]) / ([IMP] + [Ino] + [Hx]) \}$$

A faster test was developed to enable the use of nucleotide degradation indexes in quality control. These rapid test kits are available to measure K', which is calculated as shown below (O'Keefe, 2000)

K' value = [Hx] / ([IMP] + [Ino] + [Hx])

Oxidative rancidity

Many aquatic species have relative high lipid contents, thus oxidative rancidity can be a serious quality problem. Normally changes in texture (toughening), undesirable flavor and odor are some of the consequences of lipid oxidation during frozen storage (Botta, 1995). The development of rancidity in seafood is greatly affected by factors within the product such as the amount of unsaturated fatty acids and phospholipids and its physical distribution. Some environmental factors of relevance are temperature, oxygen concentration and exposure to light (Khayat and Schwall, 1983).

The rate of deterioration is characteristic of individual fish as well as the mode of preservation. This fact is very much related to the variability of the muscle composition (Gill, 1990). The percentage of fat present in the edible muscle plays an important role in the mode of spoilage. Gill illustrates this concept presenting the following example:

> Mackerel which contain from 10-30% fat in edible muscle (depending on season catch), often undergo extensive oxidative rancidity prior to onset of bacterial decomposition. Cod, pollack or hake, which contain less than 1% fat in edible tissue, spoil primarily through the proliferation of psychotropic bacteria of the genus *Pseudomonas* (*Altermonas*) (Gill, 1990, pp 682)

There is a large number of chemical reactions that can take place during lipid oxidation. The three most important methods of assessing oxidative rancidity are:

Peroxide value (PV). Measures peroxides and hydroperoxides which are the primary products of oxidation (Botta, 1995). Because peroxides are transient in an oxidizing sample, the PV value increases, reaches a peak, then decreases (Botta, 1995)

Thus, PV is a good indicator of early stages of oxidation. Potassium iodide solution reacts with peroxide bonds releasing I_2, which is then reacted with starch to yield a complex of blue coloration (Botta, 1995). The end point of titration is determined by loss of blue color after titrating iodine back to iodide using thiosulfate.

Anisidine (AnV). This method measures secondary products of lipid oxidation (Love, 1995). Therefore, during long-term storage the compounds of interest will increase in magnitude, reach a peak and then decline. Anisidine reacts with aldehydes to form schiff bases that absorb light at 350 nm (Botta, 1995).

Thiobarbituric acid reactive substances (TBA or TBARS). This method also measures secondary oxidation product. 2 Thiobarbituric acid reacts with malonaldehyde (and other TBA-reactive substances) forming a colored complex (Love, 1995). The test may be performed in several different ways, thus it is difficult to compare results without taking into account method of sample preparation and chemicals being used. Nonetheless it is frequently used, and can be performed using water or acid extracts of the sample, a steam distillate of the sample, or the extracted oil (Love, 1995).

Physical methods

Physical methods can be used to measure several important quality attributes. Considered "objective" methods, physical parameters can be precisely measured using instruments or physical standards (Botta, 1995). There are several instruments for quick and easy determinations to indicate the degree of spoilage of fish and shellfish (Botta, 1991). Nondestructive and fast methods are of primary importance to the food industry because they decrease the costs of sampling and duration of analysis in quality control (Botta, 1991). Textural changes of seafood products such as softening, hardening and gaping can be determined with Instron and Ottawa Texture Measuring System among

others (Love, 1988) Changes in color of seafood products such as yellowing due to lipid oxidation, loss of color intensity and color changes can be monitored with the aid of instruments such as Hunterlab, Minolta Chroma Meter and Machine Vision System If samples present non-uniform color surfaces measurement of specific spots (Minolta Chroma Meter) or just a portion of the sample (Hunterlab) will, most likely, not accurately represent the overall color of that sample Luzuriaga (1999) develop a Machine Vision System to analyze color of food sample that takes into account the whole surface of a sample The instrument is able to accurately predict the degree of melanosis in shrimp samples and to measure color changes in shrimp and salmon with storage

Changes in odor of seafood products over storage can be monitored using an electronic nose and pattern recognition techniques (Luzuriaga, 1999) It was shown that sensor readings correlate well with sensory scores and results from chemical analysis (Luzuriaga, 1999)

Changes in electrical properties of the skin and underlying tissue can be instantly measured and related to freshness of whole chilled fish (Wheaton and Lawson, 1985) The meter reading is directly related to the loss of freshness in days However, this has to be determined for each species because the electrical properties are different for each species (Wheaton and Lawson, 1985)

Sensory methods

Sensory procedures are defined as those solely dependent upon the human senses (Connel, 1990) The fish industries use all of the senses with exemption of hearing to judge quality Sight, touch, smell and taste can be used to evaluate a number of individual attributes, such as color, softness, fishy odor and musty taste, that reflect an overall quality of the product (Connel, 1990) One of the main advantages of sensory

methods is that they can give a valid idea of the impressions and opinions of the consumer about a product. In addition in general it can be said that human senses can better recognize and discriminate complexities (Connel, 1990). Some of the main disadvantages are the large variation of responses particularly with fatigue and adaptation, and the cost involved on training and running a sensory panel (Connel 1990).

There are many different sensory tests to evaluate odor of food samples. The choice for a specific test is determined by the objective (Bett and Grumm, 1994; Botta, 1995). The most common types are briefly described below.

Affective sensory tests

These are subjective tests that measure the spontaneous impression of panelists. Therefore, if the objective is the access acceptability or preference this test should be used. Normally a hedonic scale with a series of statements is used to express how much a subject likes or dislikes the sample under investigation (Botta, 1995). In the present work a 9-point hedonic scale was used to assess acceptability of consumers for appearance of raw white muscle of Gulf of Mexico sturgeon that were fed three different commercial diets. The ballot used on the test is shown on Figure A 1 (Appendix A). It is important to point out that even though numbers are not used on the ballot presented to the panelists the ratings can be readily changed to numbers, in this case the numbers ranged from 9 for like extremely to 1 to dislike extremely.

Discriminative sensory tests

These tests are applied when it is of interest to know if there is a perceived difference in sensory properties between samples. There are several tests used to determine differences between samples. Some examples are paired comparison test, duo-trio and triangle test. The only discriminative test used through out this work was the

triangle test. During this test the panelist is presented with three coded samples placed in a tray, two of them are the same, and the third one is different. The panelist is asked to try all the samples and then to identify the odd sample. Each panelist must be presented with three different comparisons. Therefore each panelist has 33% chance of correctly guessing the correct sample (Botta, 1995). A typical ballot used for triangle tests is shown on FigureA 2 (Appendix A). Since panelists are requested to try samples presented on a tray from left to right, randomization of placement of samples on the trays is necessary to avoid order effect. Randomization of the order each comparison is presented is also important. For example, if panelist 1 is presented with comparison A versus B at first, then panelist 2 should be presented with comparison B versus C, and panelist 3 with comparison C versus A. This will help to decrease the influence of fatigue on the final result of the test.

Descriptive sensory methods

These methods are used when the objective is to identify the specific level of a particular attribute (or a set of attributes) in a product. The two major methods are attribute rating and descriptive analysis (Botta, 1995). Attribute rating can be further subdivided into structured category scale, unstructured interval scale and magnitude estimation (Botta, 1995).. Descriptive analysis can also be subdivided into flavor profile method, texture profile method, quantitative descriptive analysis and free choice profiling (Botta, 1995).

The only descriptive method used in this work was unstructured interval scale. It was used to access specific attributes of cooked and smoked samples of Gulf sturgeon that were fed three different commercial diets. The method can be briefly described as a horizontal line scale (6 inches or 15 cm) usually with two anchors near the ends of the

line Each anchor is labeled with a word that reflects extremes of the intensity of the attribute under investigation (Bett and Grimm, 1994) The word reflecting the most intense aspect of the attribute should be placed on the right side of the line (Botta, 1995) Sometimes a third anchor can be placed at midpoint (Bett and Grimm, 1994) After the evaluation of a coded sample, the panelist places a vertical line at the point that best reflects the panellist's impression concerning the intensity of the attribute under investigation The position of this mark is measured with a ruler and transformed to centimeters or inches (Botta, 1995) Whereas there are no categories between the two extremes of the scale, this avoids the problem of unequal intervals between categories Different form the category scale, this type of scale will reveal information concerning the degree of intensity of the given attribute (Botta, 1995). The major disadvantage is the fact that it is more difficult to remember positions in a scale than numbers, thus training must be emphasized to reach consistent results during the analysis (Botta, 1995) Examples of unstructured interval scales can be found in Appendix A (Figure A 3, Figure A 4)

CHAPTER 3
RIGOR MORTIS

Introduction

The white sturgeon industry is a profitable business in California. During the early 90s several consumers began complaining about tough texture of White sturgeon fillets. A study carried out at University of California at Davis reported that the cause of texture problems was the extended duration of the rigor mortis process in White sturgeon (Izquierdo-Pulido et al., 1992). The researchers reported that onset of rigor in that sturgeon species can take from 72 up to 96 hours, while full resolution might not be archived before 7 days after killing (Izquierdo-Pulido et al., 1992). It seems that fresh White sturgeon fillets were reaching the consumer within a few days after killing, thus muscle was undergoing rigor after filleting the fish (Izquierdo-Pulido et al., 1992). Filleting fish before rigor has resolved can lead to shrinkage of the muscle, as well as excessive drip loss, both of which will contribute to muscle toughening. There is no information available in the literature regarding progression of rigor mortis in Gulf of Mexico sturgeon. Therefore, there was a need to determine time for onset and resolution of rigor mortis for this species in order to avoid possible quality problems related to changes in texture of the muscle. Two preliminary studies using a total of nine fish were performed to establish time of onset and resolution of rigor. Then, a study with a much

larger number of fish was carried out to determine if fish weight correlates with onset and resolution of rigor The results from the preliminary studies might not be representative of the population due to the small number of samples investigated Finally, a novel method of monitoring rigor was proposed using analysis of video camera images from fish in rigor over different intervals of time

Preliminary Experiments

Two preliminary experiments were carried out to investigate the time of onset, the time where fish reaches maximum rigor, and the time of rigor resolution The methodology used here is an adaptation of the method used to determine Rigor Index, which is one of the most widely accepted non-destructive methods found in the literature to monitor rigor progression over time in whole fish (Iwamoto et al , 1987)

Materials and Methods

As stated this method is very similar to the method used to determine Rigor Index However, there are three measurements were collected, while Rigor Index requires just one measurement per time interval The fish is held by the head while its body hangs free from the side of a table A protractor attached to the table measures the sagging angle at different intervals of time (Figure 3-1) The fish is then placed on top a board and the distance from the dorsal fin to the floor, and tail to the floor are measured with a ruler (Figure 3-2) Surfaces were leveled and tape stripe delimited the placement area to diminish error due to positioning Rigor mortis was investigated in three fish at first (weights 1 5 kg, 2 5 kg and 3 5 kg) The goal was to determine time frame of rigor in Gulf sturgeon. Fish were coded as 1RM, 2 RM and 3 RM The three measurements

(sag angle fin-floor distance and tail-floor distance) were collected 13 times for each fish over the course of 50 hours This was called preliminary experiment #1

The second experiment was a repetition of the first one, but with double the number of fish (weights vary between 2 0 kg to 3.5 kg) Every fish received one of the following codes ARM BRM, CRM, DRM, ERM or GRM At this time measurements were taken over 92 hours at 17 different intervals of time This was called preliminary experiment #2 Fish came from the same spawning year (1995) and were killed by submersion in ice water Fish were eviscerated within a few hours after removal from the tank and stored in coolers filled with ice water Coolers remained in a cold room (1-3^{0}C) at all times except when measurements were taken Water and ice were changed every 24 hours to keep fish stored in a clean environment.

The errors of the measurements were not determined. Therefore, an empirical assumption that a change to be considered significant should be of at least 33% from the initial value was postulated to analyze the data The data was organized into spreadsheets and the percentages of changes were calculated for each measurement at every interval of time If the change in a given measurement was 33% or bigger then the initial measurement, fish was considered in rigor state If the change was smaller than 33%, fish was not considered in rigor Final results are then given in hours to onset of rigor, hours of rigor duration, hours until rigor has resolved and time fish reaches maximum rigor Time of maximum rigor occurs at the point of maximum change from point at time zero

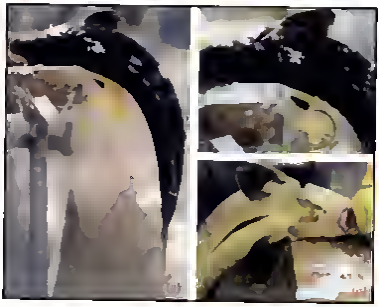

Figure 3-1 Measurement of sag angle (a) Full view, (b) Close-up right side view; (c) Close-up left side view

Figure 3-2 Measurement of fin and tail distances (a) Full view; (b) Close-up lateral view

Results and Discussion

The data from angle measurements collected over preliminary experiments #1 and #2 are shown in Table 2-1 and Table 2-2 respectively. After transforming the data to percent change from the initial value, the results were coded into a yes or no for presence of rigor mortis, and then plotted for each fish over time. Times of onset and resolution are expected to vary considerably between fish. This is expected because, as mentioned earlier, rigor is dependent on a large number of variables such as feeding habits, sexual maturation, degree of struggle, overall health of the fish, and intrinsic characteristics of each individual animal.

Table 3-1 Data of angle measurements from preliminary experiment #1 in Gulf sturgeon rigor mortis

Time (h)	Fish 1RM	Fish 2RM	Fish 3RM
3	74^0	72^0	69^0
5	74^0	73^0	71^0
10	73^0	73^0	74^0
15	73^0	73^0	73^0
20	74^0	73^0	73^0
23	73^0	73^0	74^0
26	73^0	74^0	75^0
28	75^0	72^0	75^0
33	74^0	74^0	75^0
41	80^0	78^0	79^0
44	79^0	73^0	75^0
46	78^0	74^0	76^0
50	72^0	72^0	73^0

Table 3-2 Data of angle measurements from preliminary experiment #2 in Gulf sturgeon rigor mortus

Time (h)	Fish GRM	Fish ERM	Fish DRM	Fish CRM	Fish BRM	Fish ARM
5	71°	73°	72°	70°	63°	63°
7	72°	73°	71°	70°	63°	64°
19	72°	74°	73°	79°	76°	65°
22	75°	76°	74°	80°	75°	66°
24	82°	85°	73°	78°	71°	75°
26	78°	82°	81°	74°	69°	72°
28	76°	80°	83°	75°	67°	74°
31	76°	79°	75°	73°	69°	70°
34	75°	83°	77°	72°	66°	71°
40	80°	81°	74°	72°	66°	71°
49	78°	83°	73°	70°	66°	71°
52	76°	81°	78°	71°	64°	70°
58	78°	80°	80°	71°	64°	70°
61	76°	78°	81°		64°	71°
73	75°	76°	80°		64°	70°
79	72°	75°	77°		63°	70°
85		73°	70°			66°
91			71°			62°

The data from fin floor distances are shown on Table 2-3 and 2-4 for preliminary experiments 1 and 2 respectively

Table 3-3 Data of fin-floor measurement from preliminary experiment #1 in Gulf sturgeon rigor mortus

Time (h)	Fish 1RM (cm)	Fish 2RM (cm)	Fish 3RM (cm)
0	58.3	50.8	46.7
5	58.3	50.8	46.7
10	58.4	50.8	46.8
15	58.1	50.6	46.8
20	58.4	50.6	47.0
23	58.3	50.6	47.0
26	57.9	50.5	47.3

Table 3-3 continued

Time (h)	Fish 1RM (cm)	Fish 2RM (cm)	Fish 3RM (cm)
28	58.9	50.6	47.0
33	59.1	50.6	47.0
41	62.9	54.0	48.7
44	62.9	49.5	47.5
46	62.2	50.2	47.0
50	60.3	49.5	47.0

Table 3-4 Data of fin-floor measurement from preliminary experiment #2 in Gulf sturgeon rigor mortis

Time (h)	Fish GRM (cm)	Fish ERM (cm)	Fish DRM (cm)	Fish CRM (cm)	Fish BRM (cm)	Fish ARM (cm)
5	75.9	75.4	73	72.2	70.8	69.9
7	75.9	75.2	73.2	72.2	73	69.9
19	76	75.6	73.7	77.9	74.5	70.3
22	76.5	75.9	74.0	78.1	73.8	70.6
24	78.3	78.6	73.5	77.6	73.2	73.3
26	76.8	76.7	76.8	73.3	72.7	72.4
28	76.4	75.6	77.9	73.7	72.4	73
31	76.5	76.2	74.5	73.2	73.5	73.2
34	77.5	77.0	73.7	72.4	72.1	73.2
40	80.6	81.8	74.6	72.4	73	73.3
49	78.6	81.0	73.7	72.4	72.4	74.1
52	78.1	76.5	75.9	71.6	72.9	73
58	79.1	80.0	80.0	72.1	72.4	72.4
61	77.8	75.6	80.8		72.4	73
73	76.2	75.9	76.8		73	71.1
79	75.6	75.9	73.7		72.7	71.1
85		75.4	74.0			71.6
91			73.0			69.9

The data from fin-floor distances are shown on Table 3-5 and 3-6 for preliminary experiments 1 and 2 respectively

Table 3-5 Data of tail-floor measurement from preliminary experiment #1 in Gulf sturgeon rigor mortis

Time (h)	Fish 1RM (cm)	Fish 2RM (cm)	Fish 3RM (cm)
0	78.4	73.3	69.5
5	78.1	73.5	69.9
10	78.1	73.5	69.9
15	78.3	73.3	69.9
20	78.4	73.2	70.0
23	78.3	73.5	69.9
26	78.4	73.2	70.2
28	78.9	73.0	69.9
33	78.7	73.0	69.9
41	81.9	74.9	73.0
44	81.9	71.8	70.3
46	80.8	72.4	70.5
50	79.1	71.8	70.3

Table 3-6 Data of tail-floor measurement from preliminary experiment #2 in Gulf sturgeon rigor mortis

Time (h)	Fish GRM (cm)	Fish ERM (cm)	Fish DRM (cm)	Fish CRM (cm)	Fish BRM (cm)	Fish ARM (cm)
5	54.6	55.2	50.8	52.5	46.4	45.7
7	54.6	55.0	50.8	52.5	46.8	45.6
19	55.1	55.6	51.3	56.5	51.4	46
22	55.6	55.9	50.8	57	51.4	46.5
24	57.9	59.7	50.5	55.9	48.7	51.4
26	56	58.7	54.6	55.1	47.8	50.5
28	55.4	57.2	56.0	55.6	47.3	51.0
31	56.5	57.9	52.1	54.5	48.3	47.0
34	57	59.7	53.0	53.8	47.6	48.6
40	59.7	65.4	54.0	53.3	50.2	48.1
49	62.4	62.9	55.2	54.3	48.9	49.5
52	58.1	56.5	56.2	52.1	47.0	50.5
58	58.9	78.1	78.1	51.9	46.7	58.1
61	57.2	60.6	74.1		47.3	58.4
73	55.6	55.9	58.4		46.4	47.0
79	54.3	55.7	55.7		46.4	47.0
85		55.9	52.1			48.3
91			50.8			47.9

Figure 3-3, Figure 3-4 and Figure 3-5 shows the results for each fish investigated during preliminary experiment #1. Each fish is represented by a color, and the colored bars depict duration of rigor. Notice that the red areas before and after rigor mortis are hours elapsed between consecutive measurements where there is no information on rigor

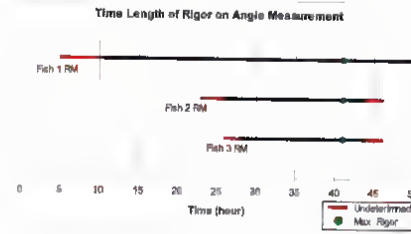

Figure 3-3 Results of angle measurements from preliminary experiment #1

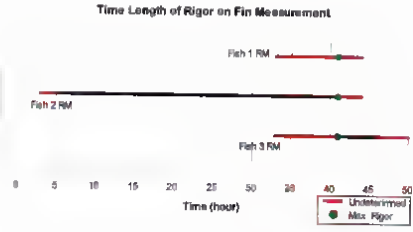

Figure 3-4 Results of fin measurements from preliminary experiment #1

Figure 3-5 Results of tail measurements from preliminary experiment #1

Figure 3-6, Figure 3-7 and Figure 3-8 shows the results for each fish investigated during preliminary experiment #2

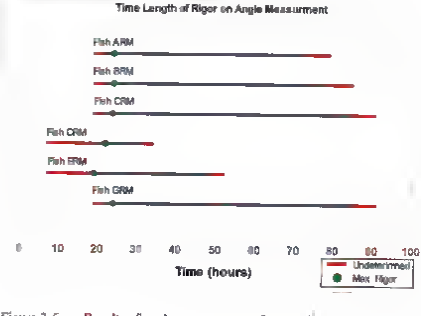

Figure 3-6 Results of angle measurements from preliminary experiment #2

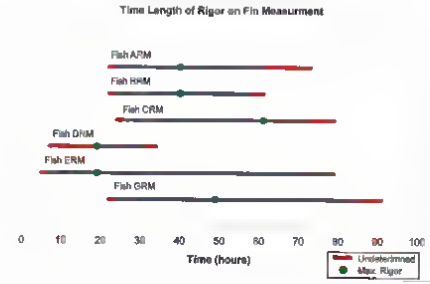

Figure 3-7 Results of fin measurements from preliminary experiment #2

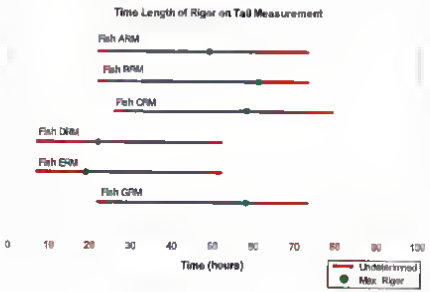

Figure 3-8 Results of tail measurements from preliminary experiment #2

Table 3-7 summarizes the results for preliminary experiment #1 and Table 3-8 summarizes the results for preliminary experiment # 2 According to angle measurement, fish 3RM was still in rigor at 50 hours Results from experiment #2 show a delay in time at which fish reaches maximum rigor between the tail and the region closer to the head (result for angle measurement) It is possible that rigor might not be uniform along Gulf sturgeon body The results are not conclusive and further investigation is needed to sustain this hypothesis Differently from White sturgeon, Gulf sturgeon take less time to enter rigor as well as to resolve it, also duration is much shorter in Gulf sturgeon Results from the present study are in agreement with research in rigor mortis of farm-raised Atlantic salmon. It was determined by Rigor Index that salmon takes an average of 8 hours to enter rigor, and between 60-70 hours to resolve it (Gill et al , 1998). Results showed that maximum muscle contraction occurred about 24-30 hours after death (Gill et al., 1998) It was pointed out that standard deviations of onset and resolution of rigor were relatively large, and that this could be due to large variation in fish size

Table 3-7 Summary of results from preliminary experiment #1

Type of Measurement	Onset (h)	Resolution (h)	Maximum Rigor (h)
Sag Angle	5-26	46-50	41
Fin Distance	3-33	44-50	41
Tail Distance	23-33	44-50	41

Table 3-8 Summary of results from preliminary experiment #2

Type of Measurement	Onset (h)	Resolution (h)	Maximum Rigor (h)
Sag Angle	19-22	31-85	23.5
Fin Distance	7-26	31-89	38
Tail Distance	19-28	49-73	44.5

Experiment 1 – Influence of Fish Weight on Rigor Mortis of Gulf Sturgeon

Materials and Methods

The experiment consisted of 50 Gulf sturgeons taken live from fish tanks at the Department of Fisheries and Agricultural Sciences at the University of Florida. All fish belonged to spawning year 1998, and were within the range of commercial size, which is between 1.5 to 4.0 kg. Fish were weighted immediately after removal from the tank, and classified according to the weight ranges below:

- Group 1 = 1.5 kg to 2.0 kg
- Group 2 = 2.0 kg to 2.5 kg
- Group 3 = 2.5 kg to 3.0 kg
- Group 4 = 3.0 kg to 3.5 kg
- Group 5 = 3.5 kg to 4.0 kg

Fish were eviscerated within a few hours after removal from the tanks and stored in coolers filled with ice water. Groups were stored in separate coolers and every fish was tagged. Every tag carried an individual number, which allowed identity to be kept throughout the study. Coolers remained in a cold room (1-3°C) at all times except when measurements were taken. Water and ice were changed every 24 hours to keep fish stored in a clean environment. Each group was composed of ten fish. Each fish was evaluated for presence of rigor over 68 hours at 8 different intervals of time. A large number of experimental units were needed in order to account for the variability between fish. However, it was not possible to run the method proposed in the preliminary experiments for 50 fish at a feasible time. Therefore, the response for the experiment was qualitative. Each fish was laid on a table and bent a couple of times in a backward and

forward movement. The fish was then held through the head and the body left to sag (Figure 3 9). The response for this experiment was a YES or NO based on stiffness. One individual performed the collection of the data at all intervals of time for every fish.

As it can be seen on Figure 3-9 there is a clear difference in body stiffness between initial point and in rigor It is important to mention that all fish were starved for a period of a week prior to killing. Starving a fish prior to killing is a preventing step to reduce the risks of contamination and also time spent during evisceration.

Figure 3 9 Gulf sturgeon and rigor mortis (a) Fish showing no rigor; (b) Fish in rigor

Results and Discussion

Figure 3-10 shows the results for the experiment Every fish has its paired response for onset (blue), and resolution (pink) The most important aspect of dealing with rigor mortis for processing fish is to ensure that fish are eviscerated and stored on ice water before it enters rigor, and that fish are not fillet before rigor has resolved Thus, by taking all the borderline points for each group of responses from Figure 3-10 and fitting linear regressions through them, it is possible to determine the time frame of rigor mortis for fish within commercial size (Figure 3-11)

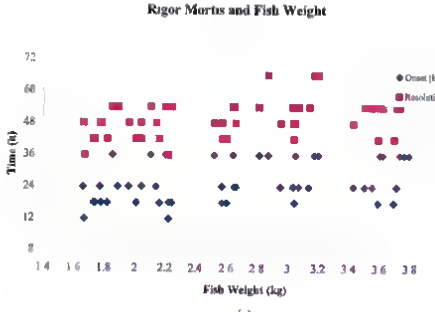

Figure 3-10 Results of study in rigor mortis and fish weight. (a) Hours to onset and resolution of rigor; (b) Regression lines fitted on minimum hours to onset and maximum hours to resolution of rigor

Its interesting to notice that the fitted lines are driven apart as fish weight increases, this means that duration of rigor mortis increases with fish weight From this data it is clear that fish within the studied range tend to start rigor within the first 24 hours after death Notice that this result is very different than the onset range of 72 to 96 hours found for White sturgeon (Izquierdo-Pulido et al , 1992) In addition, resolution of rigor in White sturgeon can take up to 7 days, while for Gulf sturgeon rigor is resolved within 3 days after death Its noteworthy to mention that reported body weight for White sturgeon investigated ranged from 1 4 to 4.5 Kg (Izquierdo-Pulido et al., 1992)

Experiment #2 - Video Capture Method for Monitoring Rigor Mortis in Whole Fish

As seen in the literature review, there are many possible ways to access rigor mortis This experiment is an improvement of Experiment 1 previously described The use of a video camera reduces the sources of error during the experiment, because measurements are not taken manually There is still possibility of variation due to placement of the fish In addition, the methodology was developed in such a way to allow some insight into whether or not rigor mortis is uniform along the fish body It is noteworthy to mention that this is a novel approach for monitoring rigor mortis in whole fish

Material and Methods

The experiment consisted of a video camera Sony Hi-8 CCD-FX710 mounted on a fixed tripod, a table and a simple apparatus to secure the fish in place that is connected to a wooden background (Figure 3-11) Three 36 inches rulers were attached to the background Eleven cultured Gulf sturgeon were killed by submersion in ice water and eviscerated within three hours of death All fish were stored in coolers filled with ice

water. Coolers remained in a cold room (1-3°C) at all times except when measurements were taken. Water and ice were changed every 24 hours to keep fish stored in a clean environment. Fish codes, sizes, weights, lengths and feeding status are presented on Table 3-1. Fish were divided into two groups: small and large. These two groups were further sub-divided into two other groups starving and fed, as seen in Table 3-1. Fish in the starved group were not fed for seven days before the first day of the experiment whereas the fed fish were fed up until the day of the experiment. The camera tripod was firmly taped to the ground and there was no change in the positioning of the camera or distance between the board and the camera during the experiment. The board was attached to the table with three large clamps. Table and board were leveled with two levers. Rulers were attached to the board with Velcro. Each fish was filmed for approximately 10 seconds at ten intervals of time over 67 hours. Therefore, the results from the experiment are ten video frames per fish. Fish were eviscerated within a few hours of death, and the experiment started approximately four hours after removal of the fish from the tanks. The first interval of time that frames were grabbed is considered time zero or zero hours. Times of data collection were 0 h, 13 h, 21 h, 27 h, 31 h, 36 h, 46 h, 52 h, 59 h, and 67 h. At 0 and 67 hours, fish were inspected for rigor and the elasticity of the bodies tested as in experiment #1; there was no indication of rigor mortis in the fish at these times.

Figure 3-11 Dimension of the apparatus used during video capture method for monitoring rigor mortis in whole fish

Table 3-9 Information on experimental units used for monitoring rigor mortis with video capturing method

Fish Code	Size	Weight (Kg)	Length (cm)	Feeding Status
RBF	Large	10 69	120	Fed
OBF	Large	8 77	115	Fed
YBF	Large	7 61	99	Fed
GBS	Large	7 66	106	Starved
PBS	Large	5 25	95	Starved

Table 3 9 continued

Fish Code	Size	Weight (Kg)	Length (cm)	Feeding Status
GSS	Small	0 64	55 5	Starved
PSS	Small	0 51	48	Starved
WSS	Small	0 48	50	Starved
RSF	Small	0 80	54	Fed
OSF	Small	0 62	53	Fed
YSF	Small	0 47	48	Fed

After the experiment was completed, one frame out of each ten seconds movie was grabbed with a video capture card on a PC The information for all grabbed images are listed below

- Width = 608 pixels
- Height = 456 pixels
- X and Y dpi = 96
- Original File Size = 831 Kbytes
- File Type = 24-Bit RGB Color
- Original File Format = Windows Bitmap (BMP)

Every frame was then modified using Corel Photo-Paint (Version 7 373) and 18 dots were placed along the fish body The coordinates of every dot were recorded The coordinate (0,0) corresponds to the middle point of dots 1 and 2 shown on Figure 3 12 Points 3 - 4 and 5 - 6 correspond to 10 inches of the rulers attached to the board. In the ruler from Corel these distances are equivalent to 1.32 inches Notice that this conversion factor is relative to the size of the image and resolution, which were determined when capturing the images from the video camera to the PC The X and Y-axis are depicted in Figure 3-12 Both x and y values are assumed to be positive, thus the plots of (X,Y)

values describing the shape of the fish body will look as if the fish were up side down (Figure 3-13) In order to have (X,Y) coordinates in real inches a conversion factor of 7 58 was multiplied by the (X,Y) coordinates of all points determined in Corel Photo-Paint. The sets of 18 (X,Y) points from every frame were organized into spreadsheets by fish, and several parameters were calculated with the aid of a computer software Rigor Program Version 1 0 6 developed by Murat Balaban (Food Science and Human Nutrition Department at the University of Florida)

Figure 3-12 Modified frame showing 18 dots that represent the shape of fish GBS at 46 hours

Figure 3-13 is a plot of all (X,Y) coordinates in inches of a given fish at time point 46 hours A diagonal line connects the first point (head point) and last point (tail

point) in the curve The perpendicular distance between the diagonal and every (X,Y) point is calculated and the values compared The largest value calculated is then assigned as D, which is one of the parameters that will be used to describe a frame Another parameter calculated was the area between the diagonal and the curve Area is calculated by two different methods, named Method #1 and Method #2.

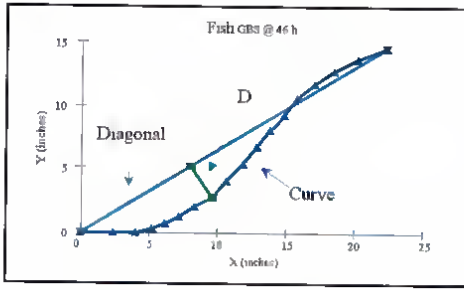

Figure 3-13 Definition of parameter for analysis of rigor mortis progression

The area between the curve and the diagonal is equivalent to the sum of the area of each slice shown on Figure 3-14 (Method #1) Note that the area of each slice is calculated by applying the trapezoidal rule

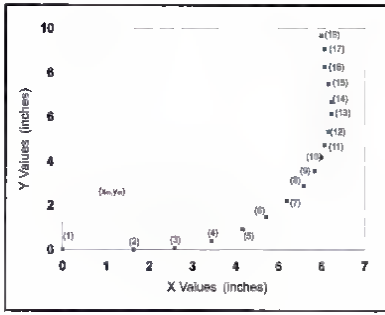

Figure3-14 Representation of calculus of area for Method #I

If the curve described by any given set of 18 (X,Y) points intersects the diagonal Method #I is not used. In this case the area over the diagonal has to be subtracted from the area under the diagonal (Area Method #2) For that, the area of the triangle composed by the X-axis, the diagonal and the red line is calculated (Figure 3-15) Then, the area under the blue curve is numerically integrated (Figure 3-15) The net area corresponds to the area of the triangle minus the area numerically integrated

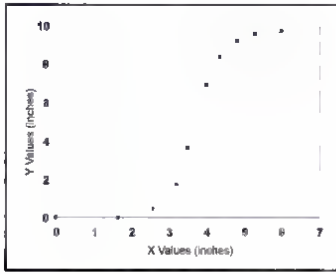

Figure 3-15. Representation of calculus of area for Method # 2

Placing a dot at the same spot six times and recording the coordinates for every try determined the error of placement of the dots in Corel Photo-Paint. This procedure was done for several frames of small and large fish. The size of the brush used on Corel Photo-Paint to draw the dots was different for small and large fish. Large fish received dots size # 5 while small fish received dots size #3. Therefore, there is a difference in the calculated error among the groups. A grid was used over every frame at about 0.1 of an inch during the process of placing the dots. It was determined that all dots fell within the orange region shown of Figure 3-16 for the fish belonging to the large group. Thus, the error for each x value and for each y value is (+/-) 0.315 inches for large fish (Figure 3-16). Leading to an error of (+/-) 0.446 inches for the parameter D (Figure 3-16). The group of small fish had (X,Y) errors estimated as (+/-) 0.158 inches, thus the error for the parameter D was calculated as (+/-) 0.223 inches (Figure 3-17).

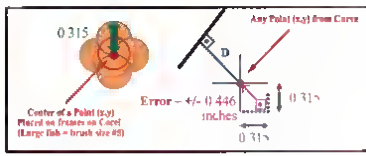

Figure 3-16 Graphic representation of estimate of error for large fish

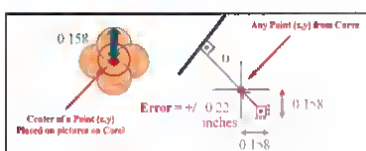

Figure 3-17 Graphic representation of estimate of error for small fish

The error of the area between the curve and the diagonal was not estimated. It is believed that this error should be small because it is dependent on the probability of misplacing each pair of (X,Y). Note that the misplacement of any given (X,Y) point might increase the error of the area or decrease it. The contribution to the area error of the error of each point will be relative to the error of the other 17 points in the curve. It can be seen that calculation of the error of area is not a straightforward task. Observe that if the errors were exactly the same for all coordinates the error of the area would be zero. The length of the curve described by the collection of 18 (X,Y) points was also calculated. Using this length, (X,Y) pairs were estimated for specific lengths of the curve. Values for (X,Y) were determined for every frame at 33% of the length of the

curve and 66% of the length of the curve These where compared to the last point of the curve called tail point or point at 100% of the curve length If the results in terms of rigor onset and point at maximum rigor are different for different lengths of the curve, it can be stated that rigor mortis is not uniform along the fish body

Finally, it is assumed that points within the error ranges are considered the same, and points falling out of the region of the estimated errors are significantly different

Results and Discussion

The results for each coded fish will be discussed separately, and then summarized for statistical analysis Fish size and starvation will be tested in order to determine if these parameters influence the progression of rigor mortis in Gulf sturgeon

Fish GBS

Figure B 1 (Appendix B) shows the frames grabbed from fish GBS during the experiment. Figure 3-18 depicts all (X,Y) points determined in Corel at the ten different intervals of time over 67 hours This fish belonged to the large group and it was starved for 7 days prior to death. Note that the curve representing (X,Y) values at 46 hours is a case where the curve intercepts the diagonal and the area is then calculated by Method #2 The results for area and parameter D are shown on Figure 3-19 and 3-20 respectively Notice that the patterns over time of the parameters are very similar The area changes about 70 inches^2 through rigor (Figure 3-19) and this corresponds to about 70% change between the initial area value, and the area value at maximum rigor This indicates a drastic change in body shape between rest and maximum rigor During the course of this experiment, it was noticed that some fish present drastic changes in body shape during rigor while others present rather small changes Note that all fish were killed by the same method The degree of struggle was similar for all fish In addition,

all fish were stored over the same conditions (temperature of storage plays an important role in the progress of rigor mortis) Thus, it seems that other factors such as starvation may play an important role in determining how intense rigor mortis process is in a fish

Figure 3-20 has the time for onset assigned as the orange point at 27 hours, rigor maximum occurs at 46 hours (red point) and resolution at 67 hours represented by the green point

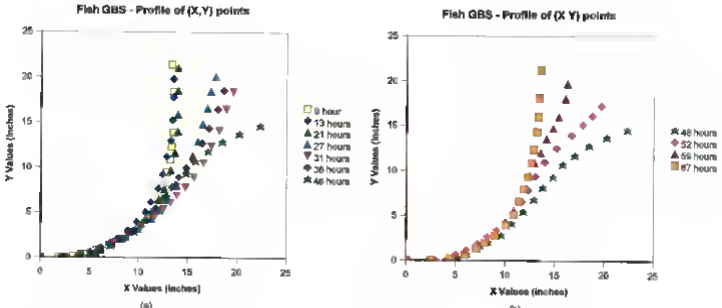

Figure 3-18 Profile fish GBS (a) (X,Y) Points up to rigor maximum
(b) (X,Y) Points after rigor maximum

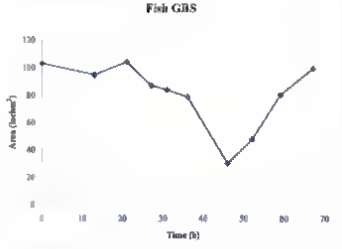

Figure 3-19 Profile of area under the curve over time for fish GBS

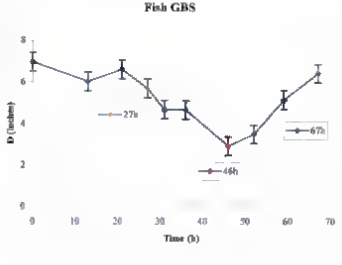

Figure 3-20 Profile of distance D over time for fish GBS

The predicted (X,Y) points for 33% of the curve length did not vary for any fish over error limits. It can be seen from Figure 3-18 that the first half of all the curves overlap. This explains the lack of difference for (X,Y) values at 33% of the curve length. This was observed for every fish, thus results for 33% of the curve length are not going to be presented. The predicted (X,Y) points for 66% of the curve length are shown in Figure 3-21. Figure 3-22 is a plot of the tail point, or 100% of the curve length. There is a difference in the point of rigor maximum between the region at 66% of the length of the fish body and the tail region. Rigor maximum is achieved at 31 hours at a point located at 66% of the curve length, but it will just be achieved at 46 hours on the tail point. This supports data of preliminary experiment #2 where it was noticed that full rigor was achieved first around the fin area and later on in the tail area. Onset and resolution of rigor occur at 27 hours and 67 hours respectively for both regions. This is in agreement with results observed for parameter D.

The error bas shown on Figure 3-21 and Figure 3-22 are suppressed for clarity from these types of graphic for all the other fish. The arrows in red represent the trajectory of the point from 0 hour up to rigor maximum. The arrows in green represent the trajectory of the point from rigor maximum to 67 hours, which was the last time point collected during the experiment.

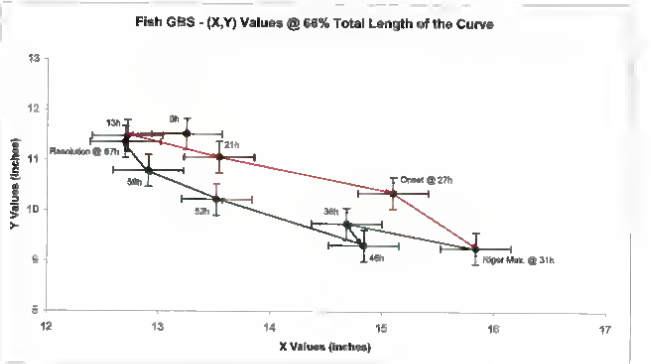

Figure 3-21 Fish GBS (X,Y) Values at 66% of total length of the curve

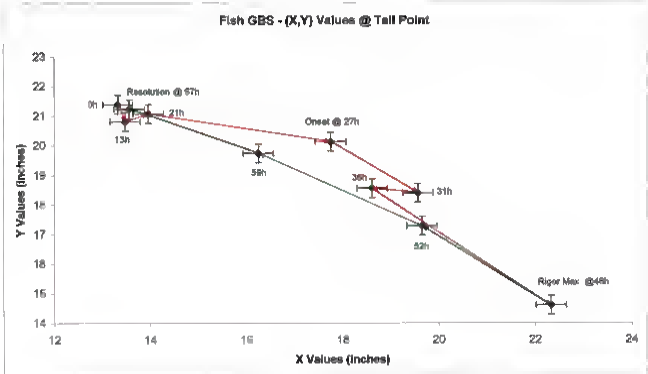

Figure 3-22 Fish GBS. (X,Y) Values at tail point

Fish PBS

Figure B-2 (Appendix B) shows the frames grabbed from fish PBS during the experiment. Figure 3-23 depicts all (X,Y) points determined in Corel at the ten different intervals of time over 67 hours. This fish belonged to the large group and it was starved for 7 days before death. The area decreases more than 30 inches^2 between point at rest and maximum rigor (Figure 3-24), this corresponds to 35 % change in area during rigor. The pattern of parameter D and area are similar over time (Figure 3-25). Parameter D varies just a couple of inches during the process, and the time for onset seems to be the same as rigor maximum at 27 hours (Figure 3-25). Parameter D suggests that resolution occurs at 46 hours for fish PBS. The predicted (X,Y) points for 66% of the curve length are shown in Figure 3-26. Figure 3-27 is a plot of the tail point, or 100% of the curve length. Notice that the (X,Y) point at 66% of the curve length does not vary much in position over time. On the other hand, there is a large variation in position over time for the tail point. Results are the same for both plots as far as rigor onset and maximum rigor, occurring respectively at 21 and 27 hours. Results for parameter D show a delay in onset when compared with results of points at 66% and 100% of the curve length. Looking at the difference between frame at 0 hour and Frame at 21 hours, it seems that fish PBS is already in rigor at 21 hours (Figure B-2). Thus parameter D fails in predicting onset. Additionally, it also failed in predicting resolution, because from Figure B-2 rigor can still be noticed at 46 hours. Resolution occurs at 52 hours for point at 66% of the curve length and just at 67 hours for the tail point. Looking at the frames at 59 and 67 hours (Figure B-2), it seems that rigor resolves at 67 hours.

Figure 3-23 Profile Fish PBS. (a) (X,Y) Points up to rigor maximum.
(b) (X,Y) Points after rigor maximum

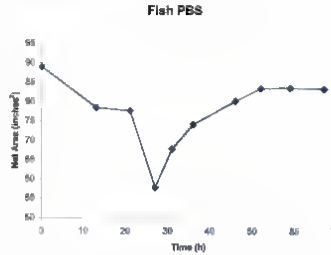

Figure 3-24 Profile of area under the curve over time for fish PBS

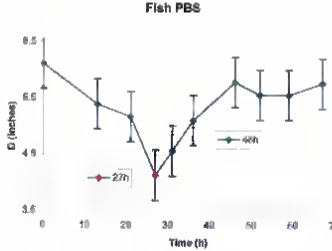

Figure 3-25 Profile of distance D over time for fish PBS

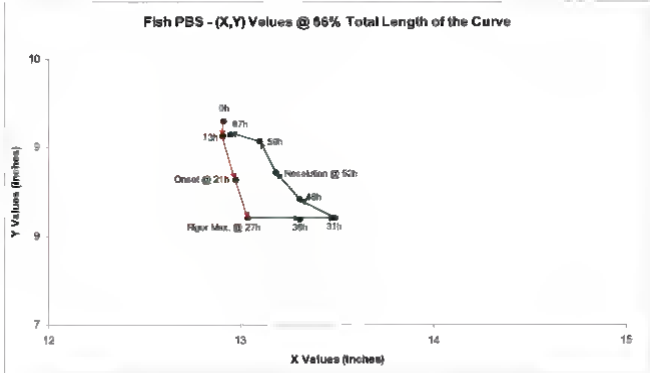

Figure 3-26 Fish PBS (X,Y) Values at 66% of total length of the curve

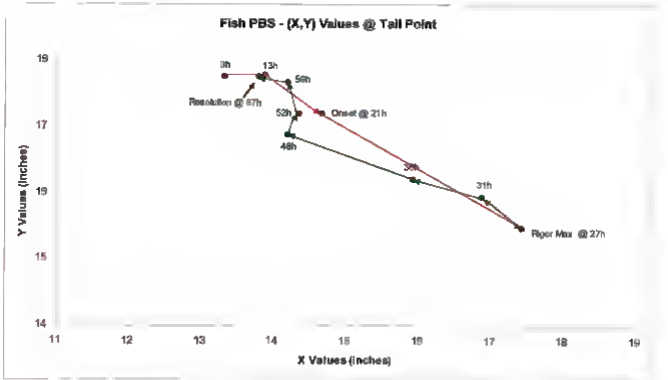

Figure 3-27 Fish PBS (X,Y) Values at tail point

Fish WSS

Figure B-3 (Appendix B) shows the frames grabbed from fish WSS during the experiment. Figure 3-28 depicts all (X,Y) points determined in Corel at the ten different intervals of time over 67 hours. This fish belong the group of small fish and it starved for 7 day before death. Notice that frame at 36 hours is atypical, as if a relaxation occurred in the fish body between two peaks of intense rigor (Figure B-3). According to results for parameter D, rigor onsets at 31 hours, rigor maximum occurs at 46 hours and rigor is resolved at 67 hours (Figure 3-29). It is clear from Figure B-3 that fish WSS is already in rigor at 27 hours thus parameter D failed to predict when rigor sets in.

The pattern of parameter D and area are similar over time, and this was noticed for most fish studied (Figure 3-30). The change in area between rest and point at rigor maximum is about 12 inches^2 (Figure 3-29) and this corresponds to about 35 % change in area over rigor. To compare change in area between small a large fish it will be necessary to refer to it as percentage of change based on initial area. Comparison of the results between fish will be presented when results are summarized. Parameter D shows two separate peaks of rigor on fish WSS, one at 31 hours and another at 46 hours. There is no reasonable explanation for this relaxation of the body during rigor. Nonetheless, this was also observed in other fish as it can be seen on fish GBS frames 31 and 36 hours, on fish OBF (Figure B-7) and also on fish OSF (Figure B-10).

Figure 3-28 Profile Fish WSS (a) (X,Y) Points up to rigor maximum.
(b) (X,Y) Points after rigor maximum

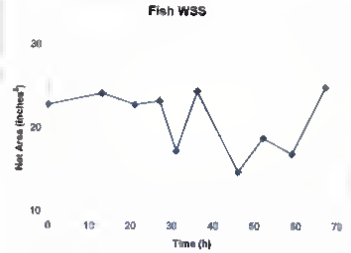

Figure 3.29 Profile of area under the curve over time for fish WSS

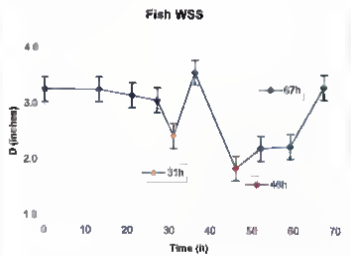

Figure 3.30 Profile of distance D over time for fish WSS

The predicted (X,Y) points for 66% of the curve length are shown in Figure 3-31 Figure 3-32 is a plot of the tail point, or 100% of the curve length Notice that point at 0 hours is dislocated to the left of the cluster of points from frame where the fish is not in rigor There seems to be a positioning difference between frame 0 and frame 13 hours because as it can be seen in Figure B-3 fish WSS is not in rigor in either of the points However, fish WSS does not show the same curvature in frames 0 and 13 hours Positioning of the fish is critical, because all results are derived from the (X,Y) points taken from the frames Rigor can definitely be noticed at 27 hours (Figure B 3) Results from graphic shown on Figure 3-31 indicate that point at 66% of the curve length failed to predict rigor onset because point at 27 hours clusters with several other points where fish is not in rigor such as points for 13 and 67 hours There is a delay in maximum rigor on the tail point (Figure 3-31) when compared to the point at 66% of the curve length (Figure 3-32). The point at 31, 46, 52 and 59 hours overlap meaning that there is no significant difference between them Thus rigor maximum was achieved at 31 hours for point at 66% of the curve length The graphic on Figure 3-32 shows two clusters The first point significantly different then 0 hours is 27 hours where rigor sets in Rigor maximum occurs at 46 hours and there is no significant change up to 52 hours because these two points clearly overlap (Figure 3-32) Observe that resolution of rigor occurs at 67 hours for all parameters investigated. Onset and resolution seem to occur at 27 and 67 hours respectively (Figure B-3), and the only parameter correctly predicting these was the tail point As in fish GBS it seems that there are two peaks of rigor, the region around 66% of the curve length peaks at 31 hours while there is still significant changes in rigor intensity on the tail area at 46 hours.

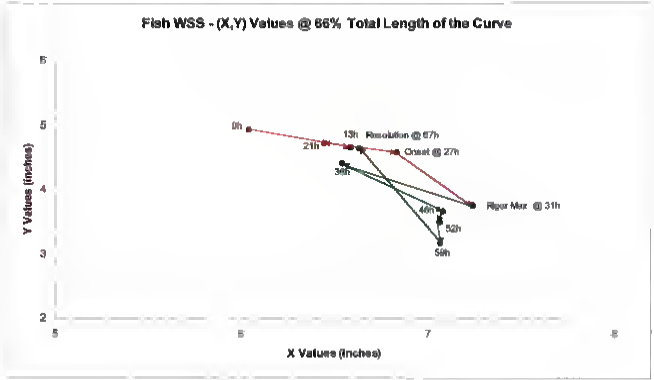

Figure 3-31 Fish WSS (X,Y) Values at 66% of total length of the curve

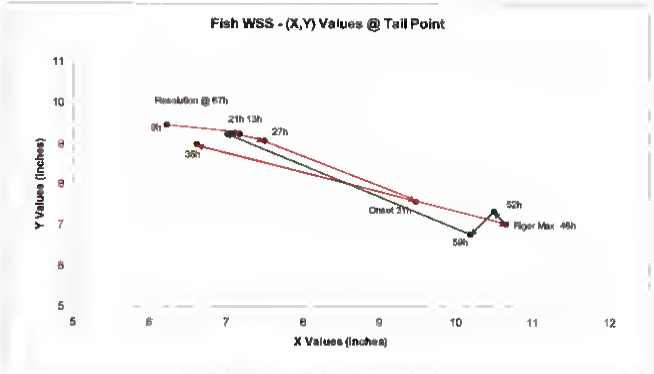

Figure 3-32 Fish WSS (X,Y) Values at tail point

Fish GSS

Figure B-4 (Appendix B) shows the frames grabbed from fish GSS during the experiment. Figure 3-33 depicts all (X Y) points determined in Corel at the ten different intervals of time over 67 hours. This fish belonged to the group of small fish and it was starved for 7 days before death. Parameter D shows rigor onset at 36 hours, maximum rigor at 46 hours and resolution at 59 hours. It can be seen on Figure B-4 that parameter D fails determining where rigor sets in as well as when it resolves. Fish at 31 hours is clearly already in rigor and fish at 59 hours is not out of rigor yet. Actually, fish GSS does not look completely out of rigor at 67 hours. The change in area is about 10 inches^2 between rest and point at rigor maximum (Figure 3-34), which corresponds to about 35 % change in area during rigor mortis. The pattern of parameter D and area are similar over time, but point 31 hours shows an increase in area, while D decreases. It is interesting to notice that an increase in area is not unexpected if rigor starts first in the region close to the head, because the tail is relaxed and will hang parallel to y axis while x values will increase when rigor sets in on the first half of the body. This causes the area within the diagonal and the curve to increase slightly instead of decreasing. Once the tail starts lifting up the size of the area between the curve and the diagonal drop significantly

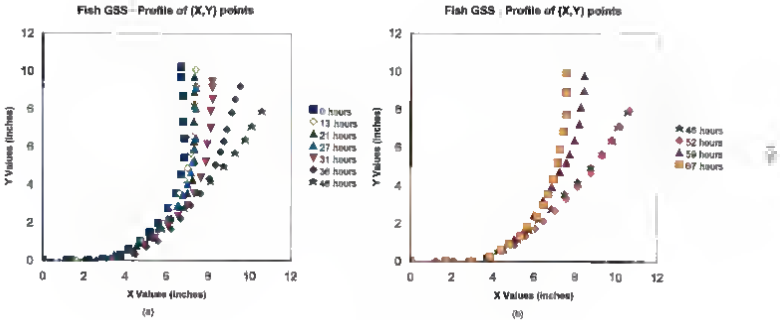

Figure 3-33 Profile Fish GSS (a) (X,Y) Points up to rigor maximum
(b) (X,Y) Points after rigor maximum

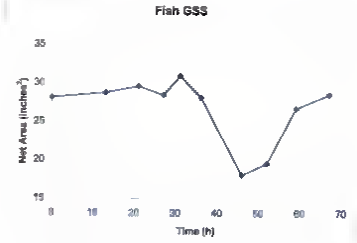

Figure 3.34 Profile of area under the curve over time for fish GSS

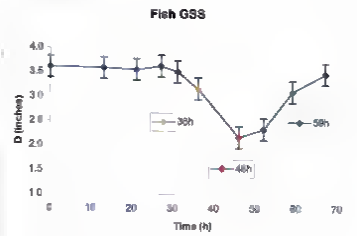

Figure 3-35 Profile of distance D over time for fish GSS

The predicted (X,Y) points for 66% of the curve length are shown in Figure 3-36 Figure 3-37 is a plot of the tail point, or 100% of the curve length. Results for onset and maximum rigor are different for the different plots. The point at 67 hours in both graphics is not overlapping with point at 0 hours, thus rigor on Fish GSS might not have been completely resolved by the end of the experiment. In Figure 3-36 it can be seen that the first point in time that does not overlap the 0 hour point is point at 27 hours, which is rigor onset according to analysis of point at 66% of the curve length. According to the tail point rigor onsets at 13 hours (Figure 3-37). It is questionable whether fish GSS is in rigor at 13 hours, but at 21 hours rigor seems to be already setting in (Figure B-4). In Figure 3-36 points at 36, 46 and 52 hours overlap so they are considered equivalent, thus rigor maximum is reached at around 36 hours. In Figure 3-37 rigor maximum occurs at 46 hours and there is no significant change up to 52 hours because these two points clearly overlap. Looking at the frames on Figure B-4, it seems that the pattern of rigor once again supports the idea that rigor intensity is different at the different regions, and the peak of rigor intensity happens at a later time for the region near the tail when compared to the region at 66% of the curve length.

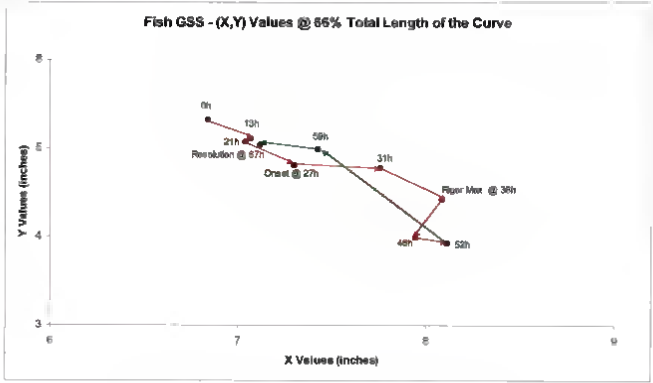

Figure 3-36 Fish GSS (X,Y) Values at 66% of total length of the curve

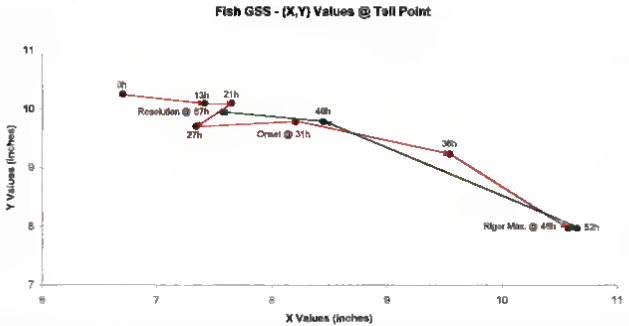

Figure 3-37 Fish GSS (X,Y) Values at tail point

Fish PSS

Figure B-5 (Appendix B) shows the frames grabbed from fish PSS during the experiment. Figure 3-38 depicts all (X,Y) points determined in Corel at the ten different intervals of time over 67 hours. This fish belonged to the group of small fish and it was starved for 7 days before death. The change in area is about 17 inches^2 between rest and point at rigor maximum (Figure 3-39), and this corresponds to 68% change in area. This is a drastic change in shape, and it indicates that fish PSS has undergone intense muscle contractions during rigor. The pattern of parameter D and area are basically identical over time (Figure 3-40). Parameter D shows rigor onset at 36 hours, maximum rigor at 52 hours and resolution at 67 hours (Figure 3-40). Looking at the frames on Figure B-5 it seems that parameter D correctly predicts when rigor sets in, as well as when it resolves. The predicted (X,Y) points for 66% of the curve length are shown in Figure 3-41. Figure 3-42 is a plot of the tail point, or 100% of the curve length. The graphs from Figure 3-41 shows two separate clusters. The first point that stands out of the cluster containing point at 0 hours, is point at 36 hours (Figure 3-41). Notice that point at 21 hours in Figure 3-42 is not clustering with points at 13 and 27 hours. Looking at Figure B-5 it can be seen that frames 0, 13, 27 and 31 are very similar and rigor does not seem to be setting in before 36 hours. Once again two peaks of rigor intensity are noticed, and rigor maximum is reached at 46 hours on point at 66% of the curve length (Figure 3-41), while tail point shows maximum rigor at 52 hours (Figure 3-42). Even though at 67 hours rigor seemed to be resolved, the tail point does not cluster with point at 0 hour (Figure 3-42).

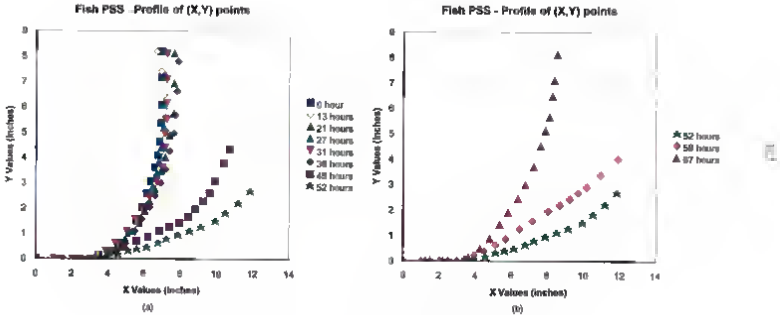

Figure 3-38 Profile Fish PSS (a) (X,Y) Points up to rigor maximum,
(b) (X,Y) Points after rigor maximum

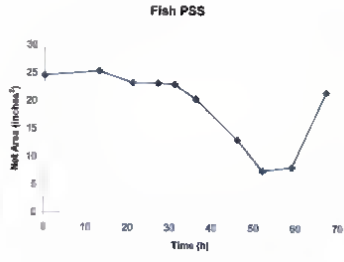

Figure 3-39 Profile of urea under the curve over time for fish PSS

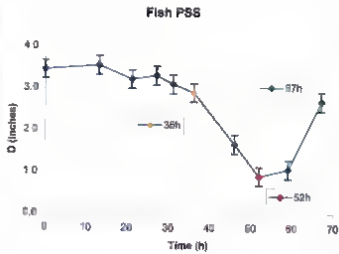

Figure 3-40 Profile of distance D over time for fish PSS

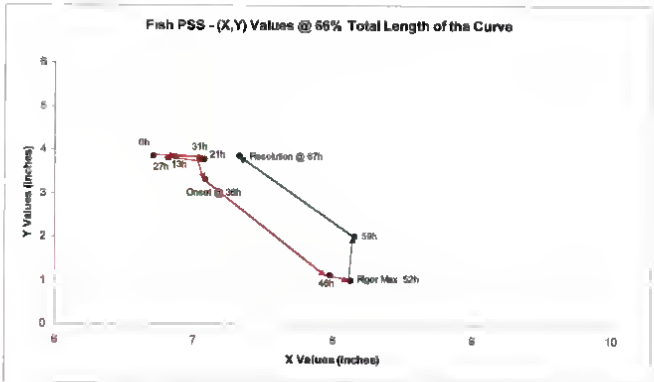

Figure 3-41 Fish PSS (X,Y) Values at 66% of total length of the curve

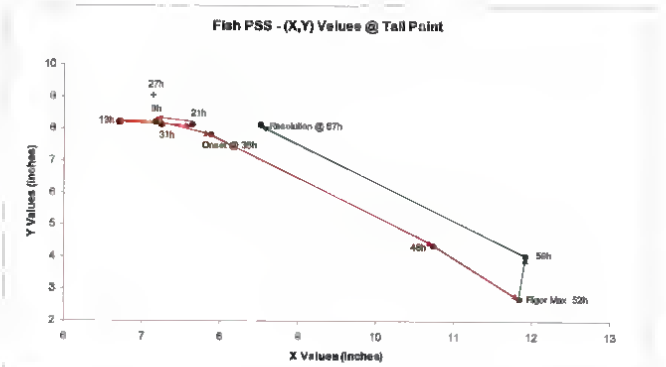

Figure 3-42 Fish PSS (X,Y) Values at tail point

Fish RBF

Figure B-6 (Appendix B) shows the frames grabbed from fish RBF during the experiment. Figure 3-43 depicts all (X,Y) points determined in Corel at the two different intervals of time over 67 hours. This fish belonged the group of large fish and was continuously fed before death. The change in area is about 30 inches^2 between rest and point at rigor maximum (Figure 3-44), and this corresponds to about 23% change in area. Notice that there is an increase in area as it was also seen for fish GBS and PSS (Figure 3-44). Looking at Figure B-6 the increase in area corresponds to onset of rigor (27 hours), where body is getting stiff but the tail is still parallel to the y axis. Tail shows full stiffness at about 59 hours. The pattern of parameter D and area are basically identical over time (Figure 3-45). Parameter D fails to predict rigor onset, and it suggests that rigor onset occurred at 46 hours. However, it can be observed that fish is in rigor since 27 hours (Figure B-6). Parameter D shows maximum rigor occurring at 46 hours, because this point is not significantly different then 52 hours. Resolution is correctly predicted at 67 hours (Figure 3-45). The predicted (X,Y) points for 66% of the curve length are shown in Figure 3-46. Figure 3-47 is a plot of the tail point, or 100% of the curve length. The graphic from Figure 3-46 shows onset at 21 hours, maximum rigor at 27 hours and resolution at 67 hours. Results from the tail point show onset at 21 hours, rigor maximum at 52 hours, and resolution at 67 hours. This is another example of a fish that shows that the tail reaches full rigor intensity at a later time than the region near the head.

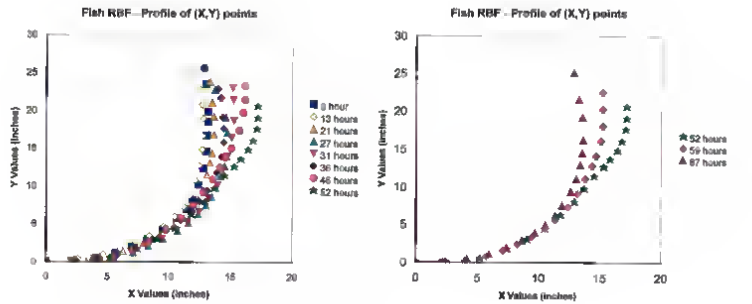

Figure 3-43 Profile Fish RBF (a) (X Y) Points up to rigor maximum
(b) (X,Y) Points after rigor maximum

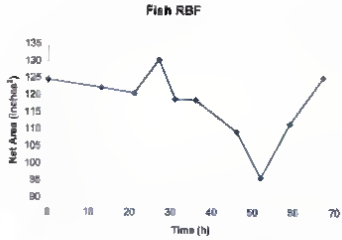

Figure 3-44 Profile of area under the curve over time for fish RBF

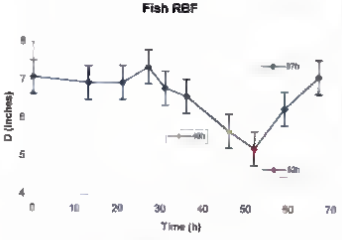

Figure 3-45 Profile of distance D over time for fish RBF

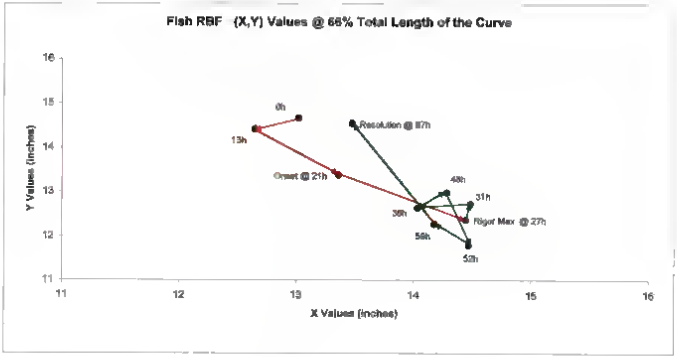

Figure 3-46 Fish RBF (X,Y) Values at 66% of total length of the curve

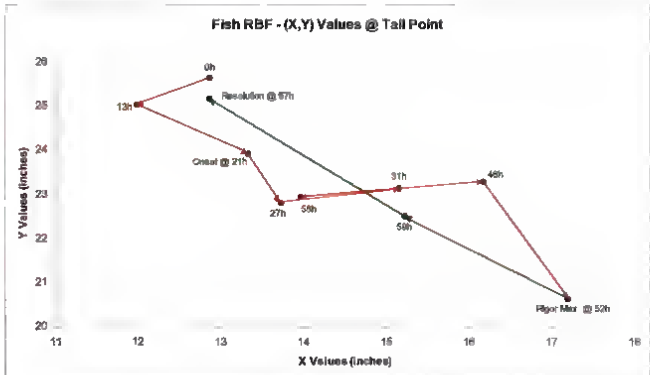

Figure 3-47 Fish RBF (X,Y) Values at tail point

Fish OBF

Figure B-7 (Appendix B) shows the frames grabbed from fish OBF during the experiment. This fish belonged the group of large fish and was continuously fed before death. The first important thing to notice is that fish is not correctly secured and in place on frame at 13 hours (Figure B-7). The fish is hanging down and badly positioned, so the frame was not included in the analysis. Figure 3-48 depicts all (X,Y) points determined in Corel at the ten different intervals of time over 67 hours. The change in area is about 17 inches^2 between rest and point at rigor maximum (Figure 3-49), and this corresponds to about 14% change in area. This indicates that there were no drastic changes in shape over time and rigor was not very intense on fish OSF. Parameter D varies about 1 inch all together and the variation is basically within the error (Figure 3-50). Parameter D in this case is of no use. The predicted (X,Y) points for 66% of the curve length are shown in Figure 3-51. Figure 3-52 is a plot of the tail point, or 100% of the curve length. The graphic from Figure 3-51 shows onset and rigor maximum at 21 hours, while tail results show onset at 21 hours and rigor maximum at 46 hours. This is another example of a fish that shows that the tail reaches full rigor intensity at a later time than the region around the middle of the body. Resolution of rigor occurred just at 67 hours on tail point (Figure 3-52). This is in agreement with Figure B-7, where fish on frame at 59 hours still shows rigor. The parameter (X,Y) at 66% of the curve length presents rather confusing results for resolution. The point at 32 hours overlap 0 hours, but subsequent points 36 and 46 hours do not overlap with 0 hours. This is another case where it is unclear why there is a 'relaxation' between peaks of rigor. Rigor resolves at 52 hours according to results from Figure 3-51. Once again the point at 66% of the curve length does not predict resolution correctly.

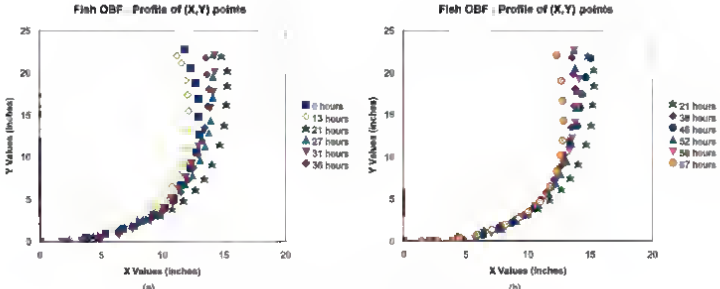

Figure 3-48 Profile Fish OBF (a) (X,Y) Points up to rigor maximum,
(b) (X,Y) Points after rigor maximum

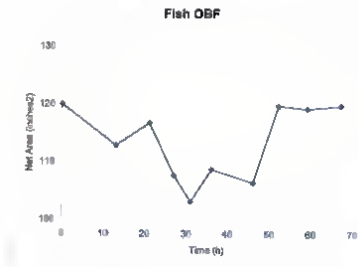

Figure 3-49 Profile of area under the curve over time for fish OBF

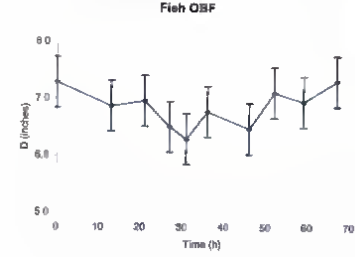

Figure 3-50 Profile of distance D over time for fish OBF

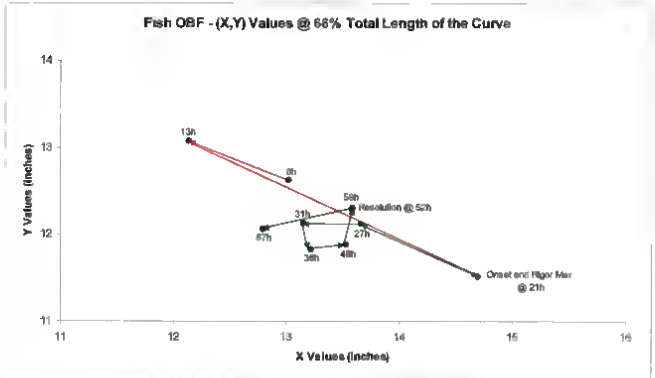

Figure 3-51 Fish OBF (X,Y) Values at 66% of total length of the curve

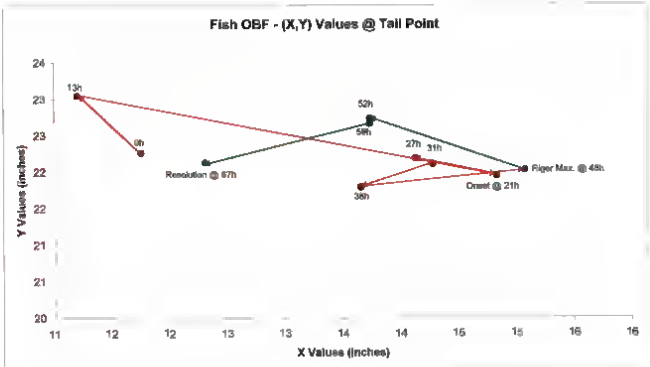

Figure 3-52 Fish OBF (X,Y) Values at tail point

Fish YBF

Figure B 8 (Appendix B) shows the frames grabbed from fish YBF during the experiment. This fish belonged the group of large fish and was continuously fed before death. The first important thing to notice is that fish show almost no rigor during the whole 67 hours. Figure 3-53 depicts all (X,Y) points determined in Corel at the ten different intervals of time over 67 hours. This fish, belong the group of big fish and it was normally fed prior to death. The change in area is about 17 inches^2 between rest and point of rigor maximum (Figure 3-54), and this corresponds to about 7% change in area. This indicates that there were slight changes in shape over time, and rigor progressed with little muscle contraction. Parameter D varies less than 1 inch over time, and the variation is basically within the error (Figure 3-55). Parameter D in this case is of no use. Curiously there are two distinct points that area drops in size, one at 21 hours and the other at 46 hours. The predicted (X,Y) points for 66% of the curve length are shown in Figure 3 56. Figure 3-57 is a plot of the tail point, or 100% of the curve length. Results from Figure 3-56 and Figure 3-57 predict onset at 13 hours, and resolution at 67 hours. The point at 66% of the curve length shows very little variation over time, and most points overlap. Point at 36 hours overlaps with points at 27 and 31 hours, thus rigor maximum occurs at 27 hours (Figure3-56). Because point at 27 hours overlaps with all points but 0 and 67 hours, it seems that rigor did not intensified after 27 hours for the region around the middle of the body of fish YBF. The results from Figure 3-57 shows that point 27 hours does not overlap with point at 36 hours, but point 36 hours overlaps with point 31 hours. This suggests that rigor maximum occurred at 31 hours at the tail point.

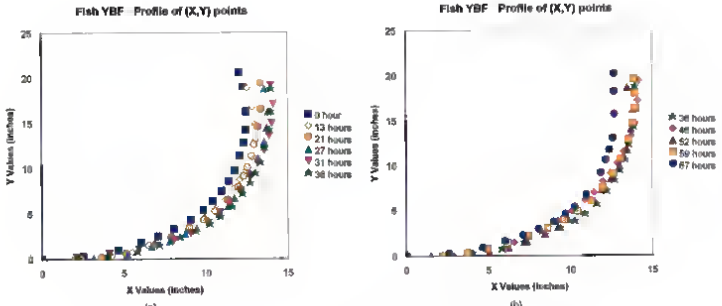

Figure 3-53 **Profile Fish YBF** (a) (X,Y) Points up to rigor maximum,
(b) (X,Y) Points after rigor maximum

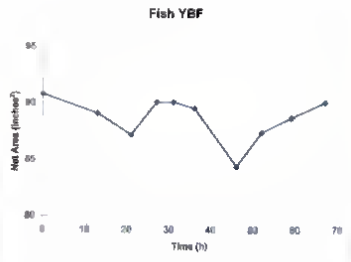

Figure 3-54 Profile of area under the curve over time for fish YBF

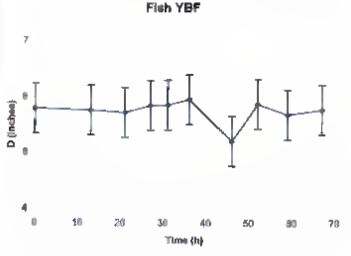

Figure 3-55 Profile of distance D over time for fish YBF

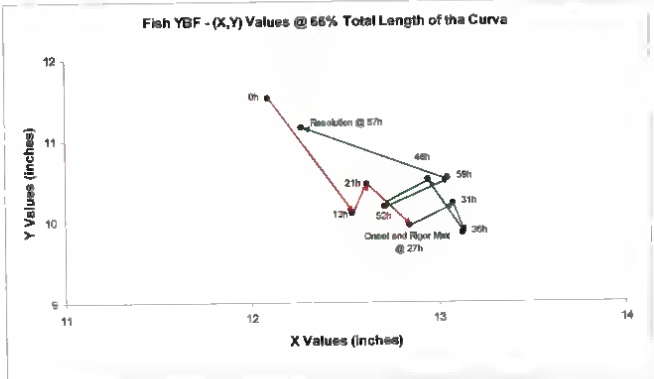

Figure 3-56 Fish YBF (X,Y) Values at 66% of total length of the curve

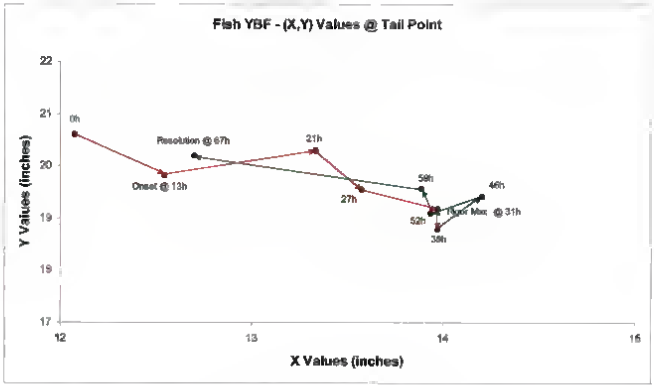

Figure 3-57 Fish YBF (X,Y) Values at tail point

Fish_YSF

Figure B-9 (Appendix B) shows the frames grabbed from fish YSF during the experiment. This fish belonged the group of small fish and was continuously fed before death. Figure 3-58 depicts all (X,Y) points determined in Corel at the ten different intervals of time over 67 hours. This fish belong the group of small fish and it was normally fed prior to death. The change in area is about 10 inches^2 between rest and point at rigor maximum (Figure 3-59) and this corresponds to about 40% change in area. This indicates that the shape of the body of this fish changed considerably during rigor. Parameter D points rigor onset at 46 hours, which is coincident to point at rigor maximum, rigor stays at maximum up to 59 hours, and resolves at 67 hours (Figure 3-60). This is exactly what can be seen on frames of Figure B-9. The predicted (X,Y) points for 66% of the curve length are shown in Figure 3-61. Figure 3-62 is a plot of the tail point, or 100% of the curve length. Results from Figure 3-61 and Figure 3-62 predict rigor onset at 36 hours and 31 hours respectively. Both points predict that fish YSF reached maximum rigor at 46 hours and resolution at 67 hours. Note that point at 31 hours does not overlap point at 0 hours, nonetheless point at 36 hours does. Fish YSF does not look in rigor at 31 or 36 hours (Figure B-9).

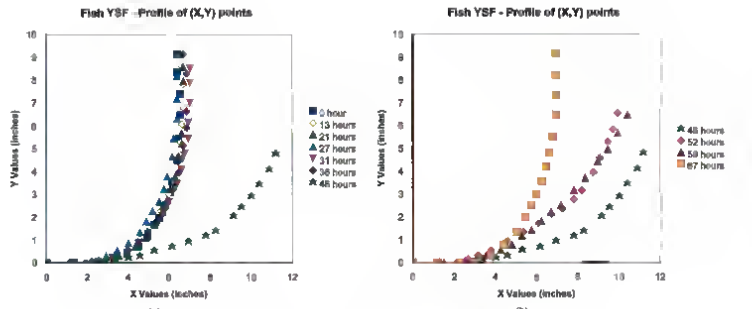

Figure 3-58 Profile Fish YSF (a) (X,Y) Points up to rigor maximum,
(b) (X,Y) Points after rigor maximum

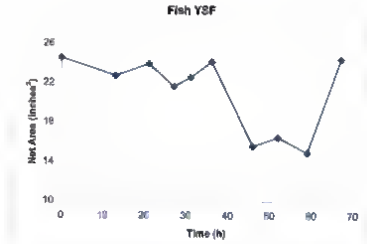

Figure 3-59 Profile of area under the curve over time for fish YSF

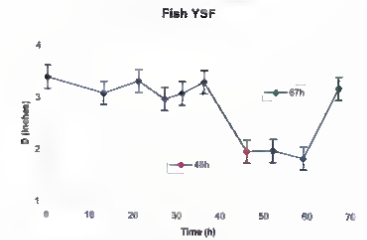

Figure 3-60 Profile of distance D over time for fish YSF

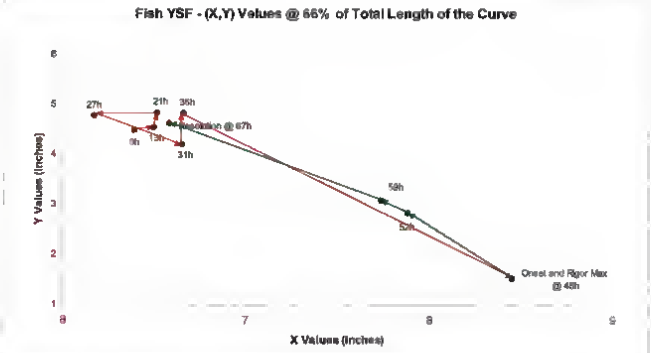

Figure 3-61 Fish YSF (X,Y) Values at 66% of total length of the curve

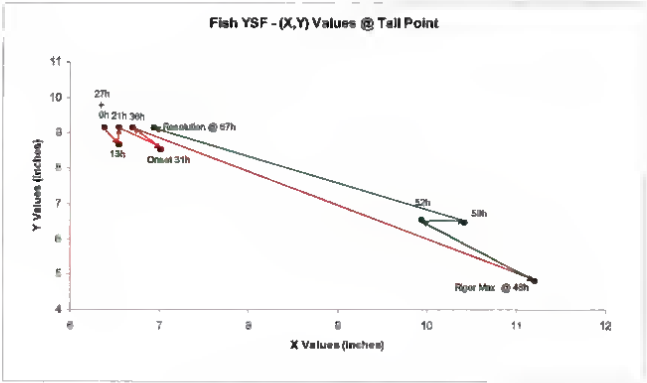

Figure 3-62 Fish YSF (X,Y) Values at tail point

Fish RSF

Figure B-10 (Appendix B) shows the frames grabbed from fish RSF during the experiment. Figure 3-63 depicts all (X,Y) points determined in Corel at the ten different intervals of time over 67 hours. This fish, belong the group of small fish and it was normally fed prior to death. The change in area is about 17 inches^2 between rest and point at rigor maximum (Figure 3-64), and this corresponds to about 60% change in area. This indicates drastic changes in shape of the body during rigor. Note that the area increases slightly then decreases. Parameter D predicts onset at 27 hours, maximum rigor at 46 hours and resolution at 67 hours (Figure 3-65). Note that curves for frames 0, 13 and 21 points are practically the same, and the first curve that stands out of this group is the one at 27 hours (Figure 3-63). In addition curve at 46 hours is clearly the point fish RSF reaches maximum rigor intensity. Parameter D in this case seems to predict well rigor progress for fish RSF. The predicted (X,Y) points for 66% of the curve length are shown in Figure 3-64. Figure 3-65 is a plot of the tail point, or 100% of the curve length. Graphic from tail point shows exactly the same times of onset, maximum rigor and resolution as parameter D. Point at 66% fails to predict rigor onset, because point at 27 hours overlaps with point at 0 hours, but maximum rigor and resolution are the same as for the other parameters.

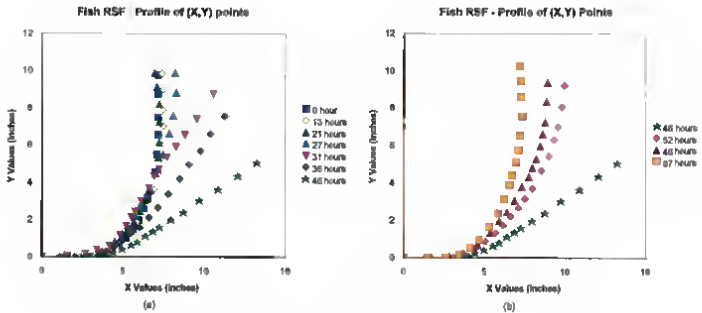

Figure 3-63 Profile Fish RSF (a) (X,Y) Points up to rigor maximum
(b) (X,Y) Points after rigor maximum

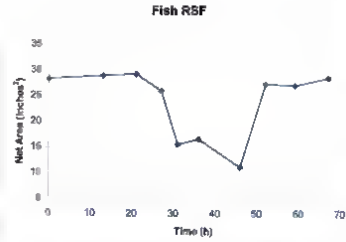

Figure 3-64 Profile of area under the curve over time for fish RSF

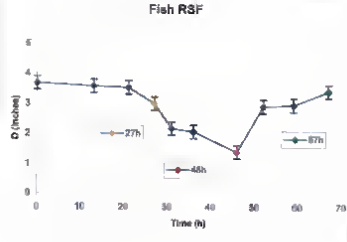

Figure 3-65 Profile of distance D over time for fish RSF

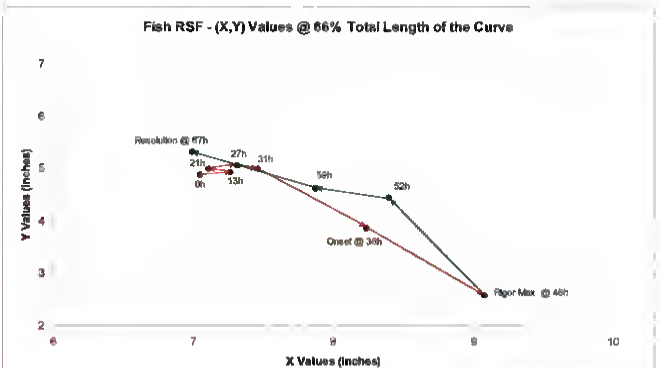

Figure 3-66 Fish RSF (X,Y) Values at 66% of total length of the curve

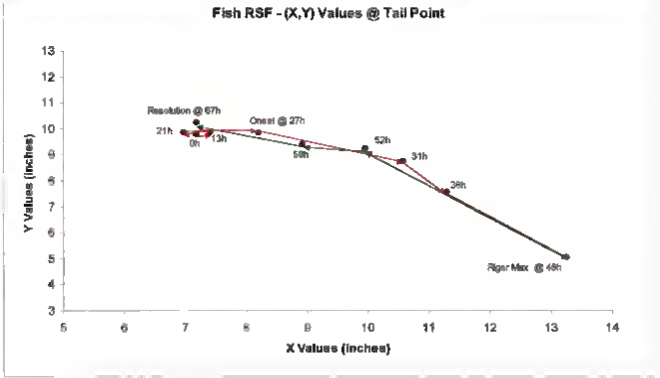

Figure 3-67 Fish RSF (X,Y) Values at tail point

Fish OSF

Figure B-11 (Appendix B) shows the frames grabbed from fish OSF during the experiment. Figure 3-68 depicts all (X,Y) points determined in Corel at the two different intervals of time over 67 hours. This fish, belong the group of small fish and it was normally fed prior to death. The change in area is less than 4 inches^2 between rest and point at rigor maximum (Figure 3-69), and this corresponds to about 14% change in area. This indicates slight changes in shape of the body during rigor. Fish YSF shows a peak of rigor at 31 hours and another peak at 52 hours, while rigor seems less intense at 36 and 46 hours (Figure B-11). Parameter D predicts onset at 27 hours, maximum rigor at 46 hours and resolution at 67 hours (Figure 3-70). Note that curves for frames 0, 13 and 21 points are practically the same, and the first curve that stands out of this group is at 27 hours (Figure 3-68). In addition curve at 31 hours is clearly the point fish OSF reaches maximum rigor intensity. Nonetheless, the tail point of curve at 52 hours sits on top of the tail point of curve at 31 hours (Figure 3-68). Parameter D varies less than an inch over time. It shows onset and maximum rigor at 52 hours, and resolution at 67 hours. It can be seen from Figure B-11 that fish is in rigor on frame 31 hours. The predicted (X,Y) points for 66% of the curve length are shown in Figure 3-71. Figure 3-72 is a plot of the tail point, or 100% of the curve length. Both parameters shows the same results: onset of rigor at 27 hours, maximum rigor at 31 hours and resolution at 67 hours. There is no explanation why frames 36 and 46 hours show a decrease in rigor intensity when compared to frames 31 and 52 hours.

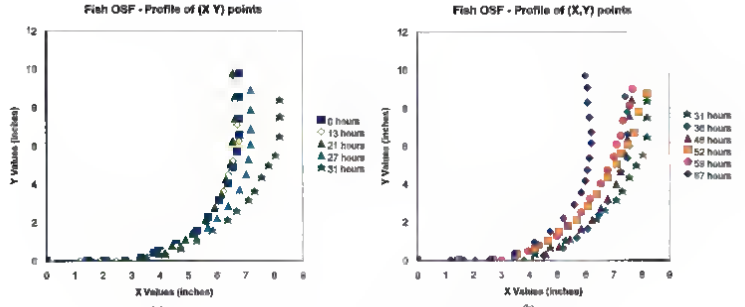

Figure 3-68 Profile Fish OSF (a) (X,Y) Points up to rigor maximum,
(b) (X,Y) Points after rigor maximum

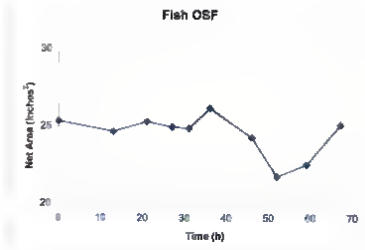

Figure 3-69 Profile of area under the curve over time for fish OSF

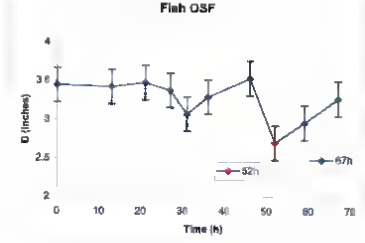

Figure 3-70 Profile of distance D over time for fish OSF

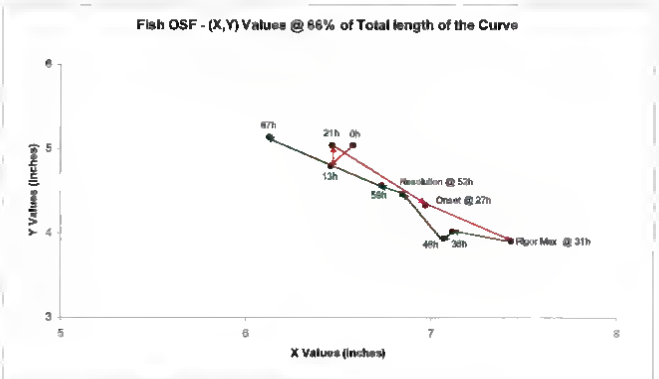

Figure 3-71 Fish OSF (X,Y) Values at 66% of total length of the curve

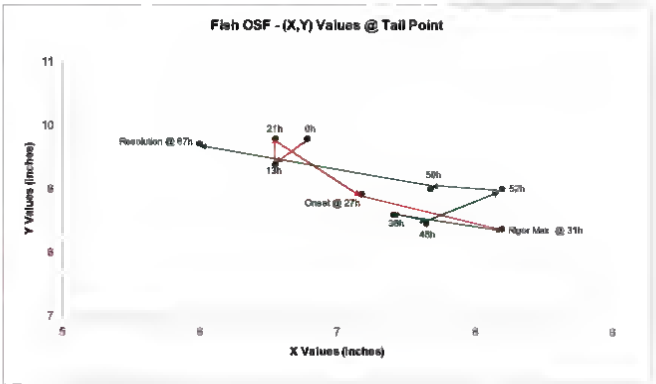

Figure 3-72 Fish OSF (X,Y) Values at tail point

Summary of Results

Before drawing conclusions regarding onset and resolution of rigor it is important to know if any of the parameters can correctly predict what is observed in the video frames grabbed during the experiment The results indicated by parameter D, point at 66% of the curve length and tail point are going to be individually compared with results from visual analysis of the frames shown on Appendix B The performance of each parameter will be evaluated Because error was not determined for the area, the results for area are going to be compared as percent change between initial frame and frame showing smaller area As discussed earlier, the overall change in area during rigor might reflect different patterns of rigor intensity between fish that were fed and fish that starved prior to death. Results of maximum rigor between point at 66% of the curve length and tail point will be compared in order to determine if rigor is uniform along the fish body, or tends to progress from the head towards the tail If any of the parameters is considered acceptable in predicting rigor onset and resolution, then the results from this parameter will be used to determine whether it is possible to differentiate groups of fish with different size and different feeding status

Parameter - area

Changes in the area are due to changes in body shape It seems reasonable to assume that if there is a lot of change in the shape of the body over rigor rigor is occurring vigorously Also, if there is little change in shape during rigor, rigor process was not intense Rigor intensity might be related to the amount of glycogen available in the fish metabolism at the time of death If ATP drops rapidly, muscle contractions should be more pronounced, and changes in shape more drastic during rigor Fish that starved prior to death should have smaller glycogen reservoir than fish that were

continuously fed, thus it should present more drastic changes in area during rigor Table 3-10 shows the initial area, and the value of the area at maximum rigor (smallest area determined) together with the percent change in area for each fish. Analysis of variance shows that there is no significant difference in rigor intensity at $p=0.05$ between starved and fed fish (Table 3-11) In addition it is expected that starvation cause fish to reach maximum body contraction in a shorter length of time due to quicker depletion of muscle plasticizers Results from analysis of variance of the time where minimum values for area were achieved show that there is no significant difference in rigor intensity between fish that starved and fish that were continuously fed prior to death (Table3-12)

Table 3-10 Summary of results from parameter area

Fish Code	Initial Area ($inches^2$)	Area at Maximum Rigor ($inches^2$)	Change in Area During Rigor (%)	Time at Maximum Rigor (h)
GBS	103.25	30.53	70.4	46
PBS	89.05	57.98	34.9	27
WSS	22.84	14.81	35.2	46
GSS	28.14	18.12	35.6	46
PSS	24.67	7.77	68.5	52
RBF	124.63	95.99	23.0	52
OBF	120.08	103.29	14.0	31
YBF	90.86	84.45	7.1	46
RSF	28.34	11.24	60.3	56
OSF	25.33	21.76	14.1	52
YSF	24.55	14.97	39.0	59

Table 3-11 Results of analysis of variance for percent change in area

Groups	Count	Sum	Average	Variance		
Starving Group	5	244 569	48 913	352 760		
Fed Group	6	157 480	26 246	400 456		
Source of Variation	SS	DF	MS	F-Value	p-value	F critical
Between Groups	1401 279	1	1401 279	3.694	0 086	5 117
Within Groups	3413 323	9	379 258			
Total	4814 602	10				

SS = sum of squares
DF = degrees of freedom
MS = Mean squares

Table 3 12 Results of analysis of variance for time at rigor maximum

Groups	Count	Sum	Average	Variance		
Starving Group	5	217	43 400	90 800		
Fed Group	6	296	49 333	99 866		
Source of Variation	SS	DF	MS	F-Value	p-Value	F critical
Between Groups	96 012	1	96 012	1 002	0 343	5 117
Within Groups	862 533	9	95 837			
Total	958 545	10				

SS = sum of squares
DF = degrees of freedom
MS = Mean squares

This result leads to the following conclusions:

- Starving Gulf sturgeon for a period of seven days might not cause a significant impact on the progression of rigor mortis
- The number of experimental units might not be large enough to show any existing significant differences
- There is almost a two-fold difference in the mean values of percent change in area between the populations, but the variance is extremely large for both groups. A larger number of experimental units could be helpful in decreasing the variance and giving a better mean separation

- The results show that starvation for a period of seven days does not cause Gulf sturgeon to reach full rigor stage faster

Parameter – distance D

Table 3-13 shows the results according to parameter D for onset and resolution of all fish but fish YBF. Results of time at maximum rigor are not going to be analyzed using parameter D because it seems that several fish present two different point of rigor maximum. Thus conclusions regarding points at maximum rigor will be analyzed using the tail point and the point at 66% of the curve length. Table 3-13 also shows the results based on a visual analysis of frames from Appendix B (observed results). Note that parameter D fails to predict rigor in all fish but fish GBS, PSS and RSF. Parameter D correctly predicted rigor resolution for seven fish out of the eleven. Parameter D is not a good indicator for rigor onset. When observing the frames it was not clear if fish GSS is already in rigor at 13 hours, as well as if RBF is in rigor at 21 hours or just at 27 hours.

Table 3-13 Summary of results from parameter D

Fish Code	D Onset (h)	D Resolution (h)	Observed Onset (h)	Observed Resolution (h)
GBS	27	67	27	67
PBS	27	46	21	67
WSS	31	67	27	67
GSS	36	59	13-21	67
PSS	36	67	36	67
RBF	46	67	21-27	67
OBF			21	67
YBF			13	67
RSF	27	67	27	67
OSF	52	67	27	67
YSF	46	67	31	67

Parameter points at 66% and 100% of curve length

First results of onset and resolution need to be compared with the observed results from frames shown on Appendix B

Table 3-14 Summary of results from points at 66% of the length of the curve (LC) and from tail point

Fish Code	66% LC* Onset (h)	66% LC* Resolution (h)	Tail Point Onset (h)	Tail Point Resolution (h)	Observed Onset (h)	Observed Resolution (h)
GBS	27	67	27	67	27	67
PBS	21	52	21	67	21	67
WSS	31	67	27	67	27	67
GSS	27	67	13	67	13-21	67
PSS	36	67	36	67	36	67
RBF	21	67	21	67	21-27	67
OBF	21	52	21	67	21	67
YBF	13	67	13	67	13	67
RSF	31	67	27	67	27	67
OSF	27	52	27	67	27	67
YSF	36	67	31	67	31	67

* LC length of curve

The parameter tail point correctly predicts onset and resolution of rigor to all fish Note that when observing the frames it was not clear if fish GSS is already in rigor at 13 hours, as well as if RBF is in rigor at 21 hours or just at 27 hours. The point at 66% of the curve length failed to predict onset in four fish out of eleven In all cases it points out onset at a later time than observed. Resolution is predicted correctly for eight fish out of eleven, and it points resolution on a earlier time than observed All fish resolve rigor between 59 and 67 hours Results for onset from the tail point were used to run analysis

of variance and show if there is a significant difference between large and small fish as well as fed and starved fish (Table 3-15)

Table 3-15. Results of analysis of variance for rigor onset from tail point (100% of the curve length)

Effect	DF Effect	MS Effect	DF Error	MS Error	F-Value	p-Value
Size	1	0.666	7	57.619	0.011	0.917
Feeding Status	1	71.185	7	57.619	1.235	0.303
Interaction	1	32.666	7	57.619	0.566	0.476

SS = sum of squares
DF = degrees of freedom
MS = Mean squares

There are no significant differences between small and large fish, or fed and starved fish. The number of experimental units is too small in each group and this might be one reason why it was not possible to show differences of rigor onset. All fish can be then treated as one group. Thus, data form maximum rigor from tail point and point at 66% of the curve length were subjected to T-test. Table 3-16 shows mean values standard deviation (SD) and standard error of the mean (SEM) for the responses on rigor maximum for both parameters. T-test shows that there is a significant difference between times of maximum rigor for point at 665 of the curve length and point at 100% of the curve length at a p-value of 0.01. This results indicates that rigor is not a uniform process, and that the tail reaches maximum rigor at a later time than the middle of the fish body. This had been observed during the preliminary experiments, but further investigation was necessary to prove it. Note that because rigor is not uniform along the fish body, determining K-values over time on muscle samples from Gulf sturgeon will require analysis of different parts of the fillets.

Table 3-16 Results of maximum rigor for points at 66% and 100% of the curve length

Fish Code	Maximum Rigor at 66% of CL (h)	Maximum Rigor at 100% of CL (h)
GBS	31	46
PBS	27	27
WSS	46	52
GSS	31	52
PSS	46	52
RBF	27	52
OBF	21	46
YBF	27	31
RSF	46	46
OSF	31	31
YSF	46	46
Means	34 5	43 7
SD	9 6	9.5
SEM	2 9	2.9
T Test	0.0136	

CL = curve length
SD = standard deviation of the mean
SEM = standard error of the mean

Conclusions

Rigor onset and resolution were determined for a large number of experimental units at commercial size This information is very valuable for the emerging aquaculture business of Gulf sturgeon in Florida In order to ensure quality of the product, fish at commercial size should be eviscerated and left to undergo rigor in ice water for the time indicated on Figure 3-11

The video capturing methodology is efficient in monitoring rigor mortis Among the parameters tested, the point at 100% of the curve length (tail point) can correctly predict onset and resolution of rigor It was possible to prove that rigor is not a uniform process in Gulf sturgeon body It is necessary to run an experiment with larger number

of fish to investigate if there are differences between fish that starved and fish that are fed continuously prior to death The proposed methodology needs to be tested for other fish species and specially for fish with body shape different than sturgeon such as eel and salmon. Finally, it is important to point out that the proposed method is non-destructive, low-cost, fast, and can be automated to fit in a production line.

CHAPTER 4
HANDLING COMPOSITION AND COMMERCIAL YIELDS

Handling Gulf of Mexico Sturgeon

There are several important aspects that should be taken into account when handling fresh sturgeon. After removal of fish from tanks, fish are placed into coolers containing water and ice. Water temperature is about 0°C thus fish are killed by submersion in cold water or during bleeding procedure. There are several other possible methods of killing such as decapitation (Watabe et al., 1991a), stunning with carbon dioxide with further placement of a cut on gill archers (Sigholt et al., 1997 Berg et al., 1997), cranial spiking (Watabe et al., 1989b; Ando et al., 1999) and sharp blow in the head (Gill et al., 1998) among others. The fish are then bled in coolers with fresh ice water. Two cuts are normally applied to the fish body (Figure 4-1). The first cut is done on the upper part of the belly cavity, aiming towards the heart. The second near the tail, to slit a vein found in that area. Fish are left in ice water for about 1-2 hours, and then gutted. Ice water should be changed at least once during the bleeding procedure. The first cut for removal of the viscera is a horizontal cut that opens the whole belly cavity without harming the organs (Figure 4-2). Viscera is removed as a whole; the belly cavity should be rinsed with clean water until clean (Figure 4-3). This is important to avoid possible contamination of intestinal cavity with bacteria. Eviscerated fish are rinsed once again and packed into coolers, or trays with clean ice water (Figure 4-4). The coolers or

trays are then stored into cold rooms held at temperature between 0-3^{0}C. The fish are left to undergo rigor at constant temperature, however ice water should be changed every 24 hours to reduce the chance of microbial contamination and ensure a clean environment for the product storage

Figure 4-1. Suggested cuts to bleed Gulf sturgeon

Figure 4-2. Gulf sturgeon belly cavity with intact viscera

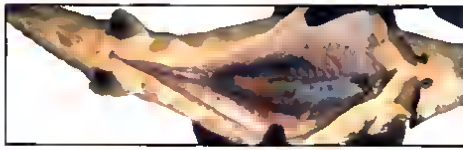

Figure 4-3. Eviscerated Gulf sturgeon

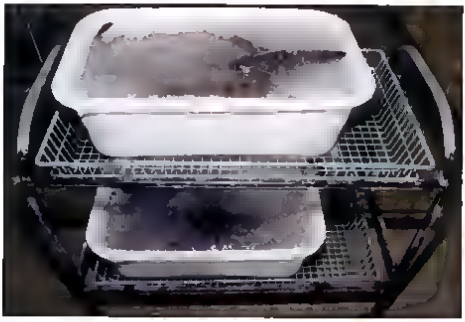

Figure 4-4 Trays with Gulf sturgeon packed in ice water

After the fish undergo rigor mortis, it should be filleted right away It is known that little bacterial contamination happens prior to resolution of rigor, but after rigor has resolved spoilage will take place even at low temperature Filleting the fish will prolong its freshness and slow down spoilage This is due to the fact that blood can still be found in several areas of the fish body regardless of the fact bleeding procedure has taken place prior to evisceration The method for filleting Gulf sturgeon described below was determined after filleting several fish different ways. Time required to fillet each fish as well as its dressed yields were taken into account to choose the best methodology

The first step in filleting Gulf sturgeon is removal of the head by placing a cut diagonal to the fish length starting right under the swim bladder and finishing under the

swim bladder on the other side (Figure 4-5 and Figure 4 6) The second step is removal of the tail as depicted in Figure 4-7 below

Figure 4-5 Removal of Gulf sturgeon head

Figure 4-6 Head-off Gulf sturgeon body

Figure 4-7 Removal of Gulf sturgeon tail

After removal of the fins, the remaining part is commercially referred as a fish bullet. Next step in filleting is removal of the belly flap. A longitudinal cut is placed from the tail area up to the head area (Figure 4-8). The two rows of bony plates located near the belly cavity are removed at this time (Figure 4-9).

Figure 4-8 Removal of Gulf sturgeon belly flap

Figure 4-9 Removal of Gulf sturgeon bottom rows of bony plates

Next a similar longitudinal cut is placed at the top of the bullet in order to remove the upper bony plate row (Figure 4-10). At this point the body is ready to be skinned. It is important to mention that under the bony plates there is a thick layer of dark skin. Using a filleting knife, the skin is loosen-up just to the lateral bony plate row, and then it is peeled off by hand (Figure 4-11). Trimming of the remaining dark skin should

decrease the chances of lipid oxidation and subsequent development of off flavors Gently sliding the filleting knife through the fat layer between the fillets the spinal cord is exposed (Figure 4-12) Note that in wild sturgeon of large size the skin is normally pulled off by hand without the need to loosen up the edges as it is shown in Figure 4-11 This is not possible in small fish because the layer of dark meat is very thin between the skin and the muscle

Figure 4-10 Removal of Gulf sturgeon top row of bonny plates

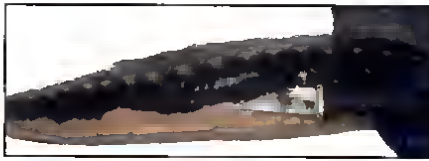

Figure 4-11 Removal of Gulf sturgeon lateral skin

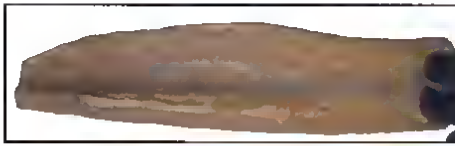

Figure 4-12 Top view of Gulf sturgeon spinal cord

Using a serrated knife to cut the spinal cord longitudinally the fillets are separated (Figure 4-13). The bones are removed by trimming, yielding 'dressed fillets' (Figure 4-14)

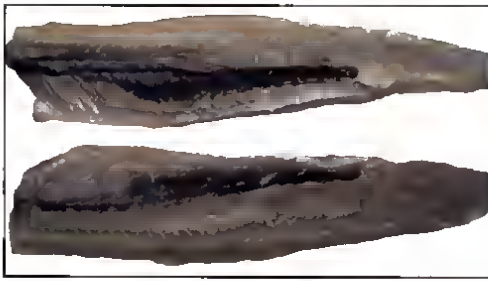

Figure 4-13 Gulf sturgeon fillets prior to trimming of belly cavity bones

Figure 4-14 Gulf sturgeon dressed fillets

Gulf of Mexico Sturgeon Proximate Composition

Introduction

According to Love (1988), when fish muscle is to be investigated for nutritional purposes, results may vary widely depending upon the part of the fillet chosen for investigation. The proportion of dark muscle increases toward the tail end, and connective tissue is minimal at about the 12th myotome counting from head to tail. Thus, it increases in the anterior and posterior direction (Love, 1988). Studies on anchovy show that the tail part presents about six time the proportion of dark muscle when compared with that near the head (Love, 1988). Japanese yellowtail has 1% dark muscle near the head, and about 57 % near the tail (Love, 1988). In addition, it has been shown that the phospholipids composition may vary widely between dark muscle and white

muscle (Love, 1988). Within the white muscle, the oil content is highest in cross-sectional slices closest to the head and least on those near the tail (Love, 1988).

Dark muscle in Gulf sturgeon should be thoroughly trimmed, as previously mentioned. Nonetheless, it is important to investigate whether there are significant variations in the proximate composition within Gulf sturgeon white muscle. This is important in case a sampling procedure is needed to quantify fat, protein, moisture and ash percent, as well as fatty acid profile, for quality control of the product.

Materials and Methods

Three live cultured Gulf sturgeons from the spawning year of 1995 were obtained from the Department of Fisheries and Agricultural Sciences at the University of Florida. All fish were killed by submersion in ice, and a slit through the heart. Fish were bled and left in ice water throughout rigor. The experimental design was as follows:

- 3 Fish = Codes 1, 2, 3
- 2 Sides = Left (L) and Right (R)
- 3 Parts = Front (F), Middle (M), and Tail (T)

Fish weights were between 2-3 Kg, and fillets weighted and average of 300-500 g each. Each fillet was equally divided into three parts; each part was stored in zip-lock freeze bags, and kept frozen at -20^{0}C until analyses.

Fillets were thoroughly comminuted to a paste in a food processor prior to duplicated analyses. Moisture and ash were determined as described in Guidelines for Chilled Fish Storage Experiments (Luna dos Santos and Tentschler, 1981). Small porcelain dishes were dried to constant weight on an oven held over night at 175^{0}C, then placed in a desicator for 30 minutes to cool down. Weights of the crucibles were recorded to four decimal places. Comminuted white muscle of Gulf sturgeon was

weighted directly into crucibles (3-5g) and total weight recorded. Samples were placed in an oven held at 105°C for 24 hours until constant weight was reached. Final weights were recorded to four decimal places, and % moisture determined by difference. Samples were then placed in a furnace held at 550°C for 24 hours or until constant weight was reached. Samples were left to cool down in desicator. Final weight was recorded to four decimal places and % ash determined by difference.

Protein content was determined by Micro-Kjeldahl (Woyewoda et al., 1986). Approximately 100 mg of sample was weighted onto weighing paper (Fisher Scientific, Pittsburgh, PA) to four decimal places. Paper and sample were placed into 100 ml digestion flasks together with 1 pellet of Kjeltabs MT (95.24% K_2SO_4 and 4.76% HgO) (Thompson Capper Ltd. Runcorn, Cheshire, England) and 4.0 ml of concentrated sulfuric acid. Samples where digested until clear (about 45 minutes). 10 ml of distilled water were added to cooled samples and then digested material was transferred to distillation unit (Rapid distillation apparatus model 65000 – Labconco, Kansas City, Missouri). 15 ml of a 40% (w/w) solution of sodium hydroxide was slowly added to the distillation flask, and 50 ml of distillate were collected into 30 ml of Kjelsorb Reagent (Fisher Scientific, Pittsburgh, PA). Titration was performed on an autotitrator (model DL12 – Mettler-Toledo, Columbus, Ohio) with 0.10 N standard HCl solution. The input parameters for the titration unit were: Speed = 1, Final pH = 5.0, Maximum HCl Volume = 50 ml, Unit # = 6 (mg/g), Constant = 87.5, and Sample Weight (mg). The unit output was % protein. Constant was calculated assuming a conversion factor of 6.25 for percentage nitrogen in the proteins combining the following equations (Woyewoda et al., 1986):

%P= (V_{HCl} x N_{HCl} x 14 007 x 100 x 6.25) / (mg of sample x 1000)

Constant =[(N_{HCl} x 14 007 x 100 x 6 5) / 1000] x 10

Instrument Result = V_{HCl} x Constant / mg of sample

Fat content was determined using a modified method of Bligh and Dyer (Christie, 1982) This method assumes sample is 80% water, thus it maybe necessary to add water to the sample 30 g of tissue were blended for 4 minutes in Waring Blendor with 100 ml of chloroform and 200 ml of methanol The mixture was filtered using a Buchner funnel, and the residue rinsed with 100 ml of chloroform The filtrate was transferred to a separatory funnel and layers allowed to separate for about 15-20 minutes. The lower layer was collected into an Erlenmeyer, dried with a few grams of anhydrous sodium sulfate, and then filtered with a regular long neck funnel using Whatman #4 filter paper The filtrate was collected into 250 ml pre-weighted round bottom flask, and solvent removed with rotatory evaporator at 50°C If lipid sample is not clear, it contains water Than 20 ml of chloroform and about 0 2 g of sodium sulfate are added The mixture filtered once again and solvent evaporated Flask and oil were weighted and lipid content determined by weight difference. Oil was stored in a freezer in a screw cap tube flushed with nitrogen

Results and Discussion

Table 4-1 shows the proximate composition, as an average value for the duplicated analysis for all samples Sides used as replicates. Analysis of variance shows that there is no significant difference between parts for lipid content and percent ash (Table 4-2) Table 4-3 shows the Means and Standard deviation (SD) of the mean for results for parts for each fish analyzed

Table 4-1 Proximate composition of Gulf sturgeon white muscle for fish spawned in 1995

Fish	Part *	% Moisture	% Ash	% Protein	% Lipids
1	F	77.45	1.09	18.40	1.03
1	F	77.55	1.08	18.55	1.22
1	M	77.15	1.10	19.50	0.94
1	M	76.80	1.11	18.70	1.17
1	T	77.35	1.05	18.60	1.02
1	T	77.30	1.08	18.80	0.93
2	F	78.65	1.11	18.00	1.46
2	F	78.70	1.13	18.00	1.26
2	M	78.45	1.00	18.10	1.34
2	M	78.75	1.15	18.05	1.10
2	T	78.55	1.10	17.55	1.09
2	T	78.60	1.07	17.40	0.98
3	F	78.00	1.04	18.60	1.57
3	F	78.00	1.10	18.50	1.22
3	M	78.35	1.01	18.15	1.06
3	M	78.35	1.06	17.85	1.05
3	T	78.30	1.02	17.80	1.07
3	T	78.60	1.05	17.70	1.13

* F=front, M=middle T = tail

Table 4-2 Results of Analysis of Variance for proximate composition data

Type of Measurement	SS Effect	DF Effect	MS Effect	SS Error	DF Error	MS Error	F- Value	p-Value
Moisture	6.41	8	0.801	0.160	9	0.018	45.070	0.00001
Ash	0.013	8	0.001	0.016	9	0.002	0.910	0.547
Protein	4.140	8	0.517	0.419	9	0.047	11.123	0.001
Lipids	0.347	8	0.043	0.167	9	0.019	2.351	0.112

SS = sum of squares
DF = degrees of freedom
MS = Mean squares

Table 4-3 Mean values and Standard deviations for proximate composition data

Fish Code - Fish Part	Moisture Means	Moisture SD	Ash Means	Ash SD	Protein Means	Protein SD	Lipids Means	Lipids SD
1 – Front	77.50	0 07	1 09	0 00	18 48	0 11	1 13	0 13
1 Middle	76 98	0.25	1 11	0 01	19 10	0.57	1 06	0 16
1 – Tail	77.33	0 04	1 07	0 02	18 70	0 14	0 98	0 06
2 – Front	78 68	0.04	1 12	0 01	18 00	0 00	1.36	0 14
2 Middle	78 60	0.21	1 08	0 11	18 08	0 04	1 22	0 17
2 – Tail	78 58	0 04	1 09	0 02	17 48	0 11	1 04	0 08
3 Front	78 00	0 00	1 07	0 04	18 55	0 07	1 40	0 25
3- Middle	78 35	0 00	1 04	0 04	18 00	0.21	1 06	0 01
3 – Tail	78 45	0 21	1 04	0 02	17 75	0 07	1 10	0 04
All Groups	78 05	0 62	1 08	0 04	18 24	0 52	1 15	0 17

Data for moisture and protein were subjected to Duncan's multiple range test for significant differences between groups Results are shown on Table 4-4 and Table 4-5 respectively

Table 4-4 Results of Duncan s multiple range test for moisture content

Fish Code - Fish Part	1 Front	1 Middle	1 Tail	2 Front	2 Middle	2 Tail	3 Front	3 Middle	3 Tail
1 – Front		0.004	0 221	0 0001	0 0001	0 0001	0 004	0 0002	0 0001
1 – Middle	0 004		0 027	0 0001	0 0001	0 0001	0 0001	0 0001	0 0001
1 – Tail	0.222	0 027		0 0001	0 0001	0 0001	0 0001	0 0001	0 0001
2 – Front	0 0001	0 0001	0 0001		0 587	0 491	0 001	0 051	0 148
2 - Middle	0 0001	0 0001	0 0001	0.587		0 855	0 002	0 113	0 309
2 – Tail	0 0001	0 0001	0 0001	0 491	0 855		0 002	0 140	0 373
3 – Front	0 005	0 0001	0 001	0 001	0 002	0 002		0 027	0 010
3- Middle	0 0001	0 0001	0 0001	0 051	0 113	0 140	0 027		0.472
3 – Tail	0 0001	0 0001	0 0001	0 148	0 309	0 373	0 010	0 472	

Table 4-5 Results of Duncan's multiple range test for ash content

Fish Code - Fish Part	1 Front	1 Middle	1 Tail	2 Front	2 Middle	2 Tail	3 Front	3 Middle	3 Tail
1 – Front		0.646	0.668	0.446	0.825	1.000	0.745	0.300	0.300
1 – Middle	0.646		0.400	0.729	0.520	0.660	0.458	0.161	0.461
1 – Tail	0.668	0.400		0.258	0.825	0.666	0.908	0.494	0.512
2 – Front	0.446	0.729	0.258		0.346	0.455	0.300	0.099	0.099
2 – Middle	0.825	0.520	0.8255	0.346		0.817	0.908	0.396	0.399
2 – Tail	1.000	0.660	0.666	0.455	0.817		0.741	0.298	0.300
3 – Front	0.745	0.458	0.908	0.300	0.908	0.741		0.446	0.455
3- Middle	0.300	0.161	0.494	0.099	0.396	0.298	0.446		1
3 – Tail	0.300	0.461	0.512	0.099	0.399	0.300	0.455	1.000	

The test pointed significant differences for protein and water content as shown in Table 4-6

Table 4-6 Results of proximate composition study on parts of fillets

	Fish #1	Fish #2	Fish #3
Moisture	F T M	F M T	F M T
Protein	F T M	F M T	F M T

The results of moisture and protein content do not show a pattern that lead to conclusions that there are real significant differences between parts On the other hand, the results seem to express differences due to variability of the measurement It is reasonable to say that there are no significant differences in the proximate composition between parts within a fillet. The range in observed moistures was 77.15 to 78 75% and in protein from 17 40 to 19 50%, for individual samples analyzed These ranges are quite small and observed significant differences reflect the precision of the measurements Thus, sampling can be applied to studies on Gulf Sturgeon raw fillets, if dark muscle is

not present Finally percent moisture has larger standard deviation suggesting that the possible differences for parts found by Duncan s test on data is due to experimental error Note that variability between fish was expected because the metabolism of each individual fish is slightly different

It can be pointed out that proximate analysis of cultured Gulf sturgeon fillets spawned over the year of 1992 were previously determined (Chen, 1993) The results shown on Table 4-7 are an average of fillets from ten fish weighting up to 3 5 kg (Chen, 1993) Protein analysis for fish spawned during 1992 was performed on a macro Kjeldhal unit that could have lead to lower recovery during the distillation step In addition, fish were fed a commercial salmonid diet in study carried out by Chen (1994), while fish were a commercial trout diet in the present study This is probably the main reason for proximate composition of fish to differ between spawning years of 1992 and 1995

Table 4-7 Results of proximate composition of Gulf sturgeon white muscle from fish spawned in 1992 and in 1995

Studies	Moisture	Ash	Protein	Lipids
Means (Chen 1994)	83 03	0 91	14 86	1 19
SD (Chen, 1994)	2 13	0 07	1 42	0 36
Means (present data)	78 05	1 08	18.24	1.15
SD (present data)	0 62	0 04	0 52	0 17

Gulf of Mexico Sturgeon Processing Yields

Introduction

Processing yields of several species of sturgeon with commercial interest have been reported (Price et al., 1989, Badiani et al , 1997) White sturgeon skinless fillets yield an average of 42 % of fish weight for fish weighting between 0 9 Kg to 3 0 Kg (Price et al., 1989) The range is rather large going from 32% up to 50% of fish weight (Price et al., 1989) The white flesh yield for Siberian and Adriatic sturgeon were reported as 58% and 55% of body weight respectively (Badiani et al , 1997) Both species range in weight from 3 to 6 Kg (Badiani et al , 1997). There were no records of Gulf sturgeon processing yields for commercial size fish found in the current literature Because farming of Gulf sturgeon will develop considerably in the near future, an experiment was set up to determine bullet and dressed fillet yields for this sturgeon species

Materials and Methods

Gulf sturgeons were taken live from culturing tanks Fish were instantly submerged in ice and eviscerated in a few hours after removal of the tank After evisceration fish were packed in coolers with ice water, and left to undergo rigor for 3 days Ice water was changed every 24 hours, and coolers were placed into a cold room at 1-3^{0}C The experiment consisted of 50 fish. Fish were removed from tank, immediately weighted, and placed into one of five weight groups Each weight group consisted of ten fish

- Group 1 Fish weight between 1.5 to 2.0 Kg
- Group 2 Fish weight between 2 0 to 2 5 Kg

- Group 3 fish weight between 2 5 to 3 0 Kg
- Group 4 Fish weight between 3 0 to 3 5 Kg
- Group 5 fish weight between 3 5 to 4 0 Kg

All 50 fish were fillet within 24 hours after resolution of rigor The filleting procedure was already described in the beginning of this chapter In order to mimic a line production, six people were involved in a step of the process of filleting One person was in charge of registering all weights. Two people were in charge of preparing the bullets, and then skinning. Two people were in charge of debonning and trimming One person was in charge of handling fish from coolers and packing the fillets in zip-lock bags that were stored on ice Fillet results are for dressed fillets or fillets ready to cook requiring no further trimming

Results and Discussion

The results for processing yields of each fish are shown on Table 4-8

Table 4-8 Results of processing yields of Gulf sturgeon

Fish Tag #	Whole Fish (Kg)	Eviscerated (Kg)	Bullet (Kg)	Fillet (Kg)	(%) Eviscerated	(%) Bullet	(%) Fillet
5552	1 96	1 79	1 26	0 41	91 28	64 25	20 91
4700	1 82	1 66	1 02	0 33	91 21	56 04	18 13
4699	1 66	1 54	0 94	0 32	92 77	56 63	19 28
4698	1 77	1 64	1 03	0 37	92 66	58 19	20 90
4697	1 67	1 56	0 98	0 33	93 41	58 68	19 76
4696	1 78	1 67	1 09	0 34	93 82	61 24	19 10
4695	1 74	1 62	1 00	0 38	93 10	57 47	21 84
4694	1 85	1 73	0 95	0 36	93 51	51 35	19 46
4693	1 73	1 61	0 90	0 39	93 06	52 02	22 54
4692	1 89	1 76	0 99	0 36	93 12	52 38	19 05
4691	2 22	2 07	1 29	0 42	93 24	58 11	18 92
4690	2 01	1 89	1 16	0 43	94 03	57 71	21 39
4689	2 04	1 91	1 07	0 48	93 63	52 45	23 53

Table 4-8 continued

Fish Tag #	Whole Fish (Kg)	Eviscerated (Kg)	Bullet (Kg)	Fillet (Kg)	(%) Eviscerated	(%) Bullet	(%) Fillet
4688	2 14	1 99	1 22	0 41	92 99	57 01	19 16
4687	2 04	1 91	1 14	0 39	93 63	55 88	19 12
4686	2 31	2 16	1 40	0 48	93 51	60 61	20 78
4685	2 20	2 04	1 28	0 35	92 73	58 18	15 91
4684	2 16	1 99	1 28	0 41	92 13	59 26	18 98
4683	2 22	2 08	1 34	0 40	93 69	60 36	18 02
4682	2 10	1 95	1 14	0 38	92 86	54 29	18 10
4681	2 57	2 37	1 61	0 57	92 22	62 65	22 18
4680	2 66	2 47	1 64	0 64	92 86	61 65	24 06
4679	2 95	2 75	1 83	0 71	93 22	62 03	24 07
4678	2 64	2 44	1 63	0 61	92 42	61 74	23 11
4677	2 52	2 36	1 67	0 72	93 65	66 27	28 57
4676	2 60	2 43	1 55	0 55	93 46	59 62	21 15
4675	2 81	2 61	1 70	0 61	92 88	60 50	21 71
4764	2 87	2 64	1 83	0 64	91 99	63 76	22 30
4673	2 57	2 40	1 56	0 54	93 39	60 70	21 01
4672	2 65	2 47	1 56	0 52	93 21	58 87	19 62
4671	3 17	2 95	1 92	0 73	93 06	60 57	23 03
4670	3 14	2 91	1 85	0 66	92 68	58 92	21 02
4669	3 04	2 85	1 78	0 59	93 75	58 55	19 41
4668	3 07	2 88	1 73	0 66	93 81	56 35	21 50
4667	3 04	2 86	1 87	0 66	94 08	61 51	21 71
4666	3 43	3 21	2 15	0 82	93 59	62 68	23 91
4665	3 05	2 84	1 72	0 60	93 27	56 49	19 70
4664	3 20	3 00	1 86	0 68	93 75	58 13	21 25
4653	3 50	3 20	1 87	0 80	91 43	53 43	22 86
4652	3 03	2 82	1 80	0 66	93 07	59 41	21 78
4663	3 60	3 38	2 33	0 91	93 89	64 72	25 28
4662	3 59	3 34	2 32	0 82	93 04	64 62	22 84
4661	3 71	3 45	2 34	0 84	92 99	63 07	22 64
4660	3 76	3 49	2 45	0 96	92 82	65 16	25 53
4659	3 69	3 44	2 44	0 83	93 22	66 12	22 49
4658	3 62	3 36	2 37	0 92	92 82	65 47	25 41
4657	3 76	3 51	2 48	0 89	93 35	65 96	23 67
4656	3 79	3 48	2 45	0 83	91 82	64 64	21 90
4655	3 73	3 47	2 29	0 94	93 03	61 39	25 20
4654	3 55	3 32	2 26	0 55	93 52	63 66	15 49

The average and standard deviations of the processing yields for each group are shown on Table 4-9 Results for % bullets and % dressed fillets are shown on Figure 4-

15 and Figure 4-16, respectively. It can be seen that there is a large variation between fish within a group, but there is a tendency of yields to get higher for heavier fish

Table 4-9 Summary of results of processing yields of Gulf sturgeon

Weight Groups	Whole Fish (Kg)	Eviscerated (Kg)	Bullet (Kg)	Fillet (Kg)	(%) Eviscerated	(%) Bullet	(%) Fillet
Means Group 1	1.79	1.66	1.02	0.36	7.20	56.83	20.10
S.D. Group 1	0.10	0.08	0.10	0.03	0.89	4.13	1.39
Means Group 2	2.14	2.00	1.23	0.42	6.76	57.39	19.39
S.D. Group 2	0.10	0.09	0.10	0.04	0.57	2.59	2.09
Means Group 3	2.68	2.49	1.66	0.61	7.07	61.78	22.78
S.D. Group 3	0.14	0.13	0.10	0.07	0.56	2.13	2.46
Means Group 4	3.17	2.95	1.86	0.69	6.75	58.60	21.62
S.D. Group 4	0.17	0.14	0.12	0.08	0.77	2.71	1.41
Means Group 5	3.68	3.424	2.373	0.849	6.95	64.48	23.05
S.D. Group 5	0.08	0.07	0.08	0.12	0.54	1.44	3.00

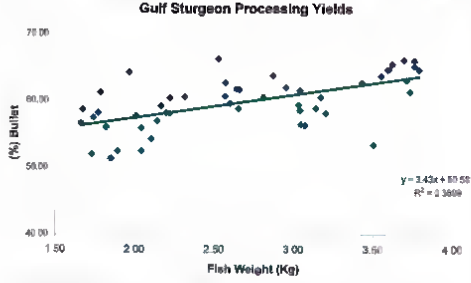

Figure 4-15 Processing yields for Gulf sturgeon bullets

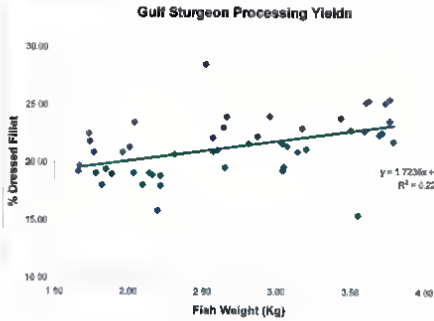

Figure 4-16 Processing yields for Gulf sturgeon dressed fillets

Conclusions

In this chapter a method of filleting Gulf sturgeon was described together with the procedure to store fish to undergo rigor mortis. A study on the proximate composition of parts of fillets of Gulf sturgeon was carried out, and the results show that there are no significant differences in the proximate composition of front, middle and tail areas within fillets. This finding is important to future studies because it shows that it is possible to run repeated measurement experiments using sampling method within fillets. Finally, processing yields of Gulf sturgeons were correlated to fish weight. Results indicate that there is a tendency of processing yields to rise with increase in fish weight.

CHAPTER 5
INFLUENCE OF COMMERCIAL DIETS ON QUALITY OF GULF STURGEON WHITE MUSCLE

Introduction

Previous research indicated that Gulf sturgeon require live feed for at least the first month of exogenous feeding before accepting of commercially available diets (Bardi, 1997). Survival and specific growth rate were extremely low for Gulf sturgeon larvae fed commercially formulated diets at the time of onset of external feeding (Bardi, 1997). Nonetheless, it was determined that larvae first feeding *Artemia* for 1 week could be switched to an experimental pelleted diet (SS9501) afterwards (Bardi, 1997). After nine months, juvenile Gulf sturgeons were fed commercially available Silver Cup trout feed manufactured by Nelson & Sons Inc. (Murray, Utah) (Bardi, 1997).

There are no commercially available diets specifically formulated for Gulf sturgeon. Therefore, a study was conducted at the Gulf sturgeon demonstration farm at University of Florida (Blowntstown, FL) to test the efficiency of three different commercially available diets. The diets used during the study were Silver Cup Trout Pellets (Nelson & Sons Inc., Murray, Utah), Hybrid Bass Pellets (Zeigler Bros. Inc, Gardners, Pennsylvania), and Catfish Fish Food Pellets (Flint River Mills, Inc. Bainbridge, Georgia). Diet composition plays an important role in the fat content and

fatty acid profile of fish muscle. It can also influence other quality parameters such as taste, texture and color, and certainly, the nutritional values of the diet in relation to sturgeon requirements will be very important. Therefore, after assessment of the growth rate, the influence of these diets on the quality of the muscle was determined. The study was designed to investigate several quality parameters. Sensory tests were carried out on cooked and smoked fillets. Consumer tests for appearance were performed on raw fillets. Color studies were conducted on raw fillets during storage at 1-3 ^{0}C. Finally, proximate composition and fatty acid profiles were determined for raw and smoked fillets.

Proximate compositions of the fish diets provided on the labels are shown in Table 5-1. The most striking difference between these diets is fat content. Catfish diet has about five times less fat than trout diet. The fatty acid profile of the diet influences the fatty acid profile of the fish muscle, so proximate composition and fatty acid profile were also determined for commercial diet used during the feeding trials.

Table 5-1. Proximate composition provided by manufacturer of the commercial diets used during feeding trials to Gulf sturgeons.

Diet Type	Crude Protein	Crude Fat (%)	Crude Fiber (%)	Ash (%)
Catfish	Not less than 33	Not less than 2	Not more than 7	Data not available
Bass	Not less than 38	Not less than 8	Not more than 4	Not more than 12
Trout	Not less than 40	Not less than 10	Not more than 3	Data not available

Materials and Methods

Gulf sturgeons were transported live from Blountstown (FL) to the Department of Food Science and Human Nutrition at the University of Florida (Gainesville, FL).

Two separate shipments of 45 fish were received within a month Each shipment was composed of 15 fish fed catfish diet, 15 fish fed trout diet, and 15 fish fed hybrid bass diet. All fish were killed in ice water, eviscerated within 24 hours after death and stored in ice water in coolers to undergo rigor mortis before filleting Coolers were stored in a cold room at 1-3^{0}C, and ice water was changed every 24 hours until rigor resolved Fish were filleted according to procedure described in Chapter 4 and yields recorded Fillets were placed in individual Ziploc bags and stored on ice prior to the beginning of the experiments Fillets were not frozen, except samples used to determine proximate composition and fatty acid profiles.

Cooking Procedure

200 ml of distilled water were added to each of the three large pans used to cook the fillets Pans were labeled for each diet. A steamer basket was placed into each of the pans and water was brought to boiling Three fillets from each treatment were steamed at a time Fillets were placed on steamer baskets and steamed for three minutes. Fillets were then turned over and steamed for an additional four minutes After initial cooling down, fillets were cut in small chunks and served while warm Note that water was discarded and pans were rinsed for every batch of three fish steamed for each one of the treatments

Smoking Procedure

Gulf sturgeon fillets were smoked according to method described in the literature with small modifications (Chapman et al., 1997) Fillets were brined in 20 L of water containing 4 Kg of sodium chloride (no iodine, Publix, FL) and 2 kg of Carnal 2110 (Gallard-Schlesinger Industries Inc , NY) When preparing the brining solution, the mixture of phosphates (Carnal 2110) is dissolved, and then sodium chloride is added to

speed up dissolution (Chapman et al., 1997). Individual brining solutions were prepared for each treatment. Fillets were brined for 45 minutes at ambient temperature (25°C-30°C) with mixing at 15 minutes intervals and then placed on screened racks (1.5 cm screen size) to dry. A circulatory fan was used to speed up drying and fillets were dried for one hour at ambient temperature (25-30°C). Fillets were placed in a standard Koch commercial smokehouse with dimensions 1m x 1m x 1.5m (height). Smoking chamber was preheated to operating temperature. Sieved hickory sawdust was used in the smoke generator (Koch, MO), and smoke was transferred to the smoking chamber using a blower. Temperature was controlled at 75°C using a thermocouple with feedback to the heater, and fillets were smoked for a total of 3 hours. Rotation of racks was performed every hour. This procedure helps smoking process to be uniform between filets. This is due to the fact that the smoke enters the smoking chamber through a pipe which is located at the back, close to the bottom of the chamber. Thus samples on the rack placed on the lowest position tend to receive more concentrated smoke than the samples on the rack placed at the highest position. Fillets were left to cool down for approximately 20 minutes and then stored in individual Ziploc bags overnight on ice in a cold store (1-3°C). Sensory panel tests were carried out on the following day. Samples used to determine proximate composition were frozen at -30°C until analysis.

Sensory Evaluation

Four sensory tests were conducted throughout this study. Sensory evaluations requiring tasting of samples were performed in the Food Science Sensory Laboratory at the University of Florida. Panelists were faculty, staff and students from the University of Florida. All participants were fish consumers.

A consumer test for appearance of raw fillets was conducted to determine if there was a visual preference for fillets belonging to a specific diet treatment. This test was conducted in the pilot plant at the Department of Food Science and Human Nutrition at the University of Florida. Six trays with dimensions 70 cm x 35 cm x 20 cm (height) were used to present samples. A layer of approximately 12 cm of crushed ice was placed in each tray. Four fillets belonging to the same treatment were placed on top of the ice layer. Each tray was coded with three digit random numbers. Trays were covered with a thin transparent plastic film. Notice that this procedure intended to mimic presentation of fish fillets at grocery stores. Two large tables were coded and three trays placed in each table, thus results from each panelist were replicated. A 9 point hedonic scale for likability was used as show on Figure A-1 (Appendix A). A total of 66 panelists participate in the study.

A triangle sensory panel was conducted to determine if there was a taste difference between steamed fillets from different treatments. Evaluation was conducted as described by Botta (1995). The ballot used for this test is shown in Figure A-2 (Appendix A). Samples were coded with random three digit numbers, and randomized for order of presentation and placement within a tray. Each panelist received one tray with three plastic cups at a time along with a plastic fork, an unsalted cracker and a cup of distilled water. Plastic cups contained two to three small pieces of fish and panelists were asked to taste samples in the order presented. Panelists were presented with a total of three trays, and were asked to take a 5-10 minutes break between evaluations. Fish were steamed right before serving, thus samples were presented warm. A total of 35 panelists participated in the test.

Two separate consumer sensory tests for specific attributes were conducted using the same methodology. The first one evaluated samples of steamed fillets of sturgeon, and the second one samples of smoked fillets. Each panelist received one tray containing three small coded plastic cups along with a plastic fork, an unsalted cracker and a cup of water. Each cup contained about 2 to 3 pieces of fish. Panelists were asked to taste samples in the order presented. Samples were randomized for order of presentation.

A total of 45 panelists evaluated the smoked samples. Unstructured scales with verbal anchors at both ends were used as it can be seen on Figure A-3 (Appendix A). The attributes chosen for this test were moisture, saltiness, fatty, smoked flavor intensity and likability.

A total of 66 panelists evaluated the steamed samples. Unstructured scales with verbal anchors at both ends were used as it can be seen on Figure A-4 (Appendix A). The attributes chosen for this test were tenderness, moisture, fishiness, rubbery, and likability.

Proximate Analysis

Fillets were thoroughly comminuted to a paste in a food processor prior to duplicated analyses. Moisture and ash were determined as described in Guidelines for Chilled Fish Storage Experiments (Luna dos Santos and Teutscher, 1981). Small porcelain dishes were dried to constant weight on an oven held over night at 175^{0}C, then placed in a desicator for 30 minutes to cool down. Weights of the crucibles were recorded to four decimal places. Comminuted white muscle of Gulf sturgeon was weighted directly into crucibles (3-5g) and total weight recorded. Samples were placed in an oven held at 105^{0}C for 24 hours, until constant weight was reached. Final weight was recorded to four decimal places and % moisture was determined by difference.

Samples were then placed in a furnace held at 550^{0}C for 24 hours or until constant weight was reached Samples were left to cool down in desicator Final weight was recorded to four decimal places, and % ash determined by difference

Protein content was determined by Micro-Kjeldahl (Woyeodu et al , 1986) Approximately 100 mg of sample was weighted onto weighing paper (Fisher Scientific Pittsburgh, PA) to four decimal places Paper and sample were placed into 100 ml digestion flasks together with 1 pellet of Kjeltabs MT (95.24% K_2SO_4 and 4.76% HgO) (Thompson Capper Ltd , Runcorn, Cheshire, England) and 4 0 ml of concentrated sulfuric acid Samples where digested until clear (about 45 minutes) 10 ml of distilled water were added to cooled samples, and then digested material was transferred to distillation unit (Rapid distillation apparatus model 65000 – Labconco Kansas City Missouri) 15 ml of a 40% (w/w) solution of sodium hydroxide was slowly added to the distillation flask, and 50 ml of distillate were collected into 30 ml of Kjelsorb Reagent (Fisher Scientific, Pittsburgh, PA) Titration was performed on an autotitrator (model DL12 – Mettler-Toledo, Columbus, Ohio) with 0 10 N standard HCl solution The input parameters for the titration unit were Speed = 1, Final pH = 5 0, Maximum HCl Volume = 50 ml Unit # = 6 (mg/g), Constant = 87 5, and Sample Weight (mg) The unit output was % protein Constant was calculated assuming a conversion factor of 6 25 for percentage nitrogen in the proteins combining the following equations (Woyewodu et al., 1986

$$\%P = (V_{HCl} \times N_{HCl} \times 14.007 \times 100 \times 6.25) / (\text{mg of sample} \times 1000)$$

$$\text{Constant} = [(N_{HCl} \times 14.007 \times 100 \times 6.5) / 1000] \times 10$$

$$\text{Instrument Result} = V_{HCl} \times \text{Constant} / \text{mg of sample}$$

Fat content was determined using a modified method of Bligh and Dyer (Christie, 1982). This method assumes sample is 80% water, thus it maybe necessary to add water to the sample. 30 g of tissue were blended for 4 minutes in Waring Blendor with 100 ml of chloroform and 200 ml of methanol. The mixture was filtered using a Buchner funnel, and the residue rinsed with 100 ml of chloroform. The filtrate was transferred to a separatory funnel and layers allowed to separate for about 15-20 minutes. The lower layer was collected into an Erlenmeyer, dried with a few grams of anhydrous sodium sulfate, and then filtered with a regular long neck funnel using Whatman #4 filter paper. The filtrate was collected into 250 ml pre-weighted round bottom flask, and solvent removed with rotatory evaporator at 50°C. If lipid sample is not clear, it contains water. Then 20 ml of chloroform and about 0.2 g of sodium sulfate are added. The mixture filtered once again and solvent evaporated. Flask and oil were weighted and lipid content determined by weight difference. Oil was stored in a freezer in a screw cap tube flushed with nitrogen.

Fatty Acid Profile

Methyl esters were prepared according to Maxwell and Marmer (1983). Around 20 mg of lipids (about 1 drop) were added to a leak-proof Teflon-lined screw cap tube and dissolved in 2.0 ml of isooctane. 200 ml of 2 N KOH in methanol (1.12g/10ml) were added and the tube vortexed for 60 s, then centrifuged. The lower layer was discarded with a Pasteur pipette. 0.5 ml of a saturated aqueous solution of ammonium acetate was added, vortexed, centrifuged and the lower layer discarded. 0.5 ml of water was added, vortexed, centrifuged and the lower layer of water discarded. A small amount of sodium sulfate was added, the tube was held at room temperature for 5 minutes, and then centrifuged. The top layer was removed and placed in a GC autosampler vial.

A gas chromatograph (model GC 14 A, Shimadzu, Norcross GA) equipped with DB225 capillary column (J&W, Folsom, CA) was used to determine the fatty acid profile. Hydrogen was used as carrier gas at a linear flow of 40 cm/sec. Injector temperature was 275°C, and oven programming was 140°C to 240°C at a rate of 4°C/min. Total run time was 25 minutes. The oven was held at 240°C for 10 minutes after the run to help clean the column between runs. Attenuation was set to 5, with a minimum area of integration equal to 500. The standards used to help identification of peaks were Supleco 37 (Supleco, St. Louis, MO), GC 68 A (Nuchekprep Inc., Elysian, MN) and cod liver oil.

Color Analysis

Color of raw fillets was determined using the Lab scale with CR-200 Chroma Meter (Minolta, Japan) and Machine Vision System (Figure 5-1). The color parameters measured are lightness (L), chromaticity of red/green (a), and chromaticity of blue and green (b) (Figure 5-2). These parameters are in accordance with the recommendations of the International Commission on Illumination, CIE (1976).

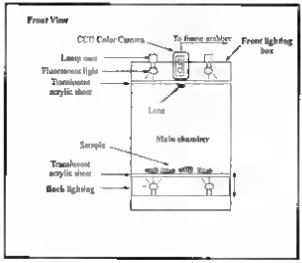

Figure 5-1 Illustration of machine vision. Source: Luzuriaga et al., 1997

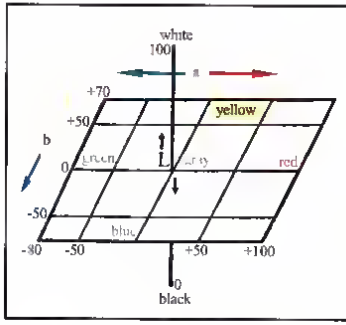

Figure 5-2. Lab color system Adapted from Francis, 1998

CR-200 Chroma Meter measures the reflectance of light from a sample surface compared to a standard calibration plate (white) Machine Vision grabs an image of the sample and Lab values are determined with the aid of computer software developed by Luzuriaga (1999). A standard orange tile #D33C-423 (Hunters Associates Laboratory Inc., Fairfax, VA) was used for calibration prior to color analysis Five fillets from each treatment were used during this experiment, and measurements were taken at 0, 5, 10 and 15 days of storage on ice with both instruments Each fillet was stored separately in a zip-lock bag that was placed in a cooler filled with crushed ice Coolers were kept in a cold room at 1-3^{0}C

Results and Discussion

Processing Yields

Yields were calculated for bullet weight, dressed fillet weight, % bullet and % dressed fillet for every fish Table 5 2 shows results of yields from fish belonging to the group fed catfish diet Table 5-3 shows results of yields from fish belonging to the fish fed bass and Table 5-4 shows results of yields from fish fed trout diet

Table 5-2 Results of yields of Gulf sturgeon fed catfish diet

Fish (Kg)	Bullet (Kg)	Dressed Fillet (Kg)	% Bullet	% Dressed Fillet
1 40	0 89	0 31	63 57	22.14
1 15	0 74	0 33	64 35	28 70
1 12	0 66	0 25	58 93	22.32
1 15	0 71	0 33	61 74	28 70
1 16	0 73	0 25	62 93	21 55
1 22	0 79	0 37	64 75	30 33
1 59	0 99	0 45	62 26	28 30
1 20	0 75	0 31	62 50	25 83
1 02	0 63	0 29	61 76	28 43
1 42	0 88	0 41	61 97	28 87
1 18	0 77	0 36	65 25	30 51
0 86	0 55	0 26	64 25	30 37
0 97	0 60	0 27	61 86	27 84
0 90	0 53	0 20	58 89	22 22
1 12	0 70	0 34	62 50	30 36
1 23	0 71	0 32	57 72	26 02
1 31	0 76	0 37	58 02	28 24
0 99	0 59	0 29	59 60	29 29
1 37	0 86	0 38	62 77	27 74
1 37	0 78	0 38	56 93	27 74
1 54	0 94	0 45	61 04	29 22
1 37	0 84	0 45	61 31	32 85
1 36	0 83	0 42	61 03	30 88
1 39	0 86	0 40	61 87	28 78

Table 5.8 Results of yields of Gulf sturgeons fed bass diet

Fish (Kg)	Bullet (Kg)	Dressed Fillet (Kg)	% Bullet	% Dressed Fillet
1.26	0.77	0.36	61.11	28.57
1.43	0.90	0.38	62.94	26.57
1.02	0.64	0.32	62.75	31.37
1.13	0.68	0.27	60.18	23.89
1.14	0.64	0.30	56.14	26.32
0.81	0.40	0.18	49.38	22.22
1.71	1.13	0.45	66.08	26.32
1.72	1.15	0.57	66.86	33.14
1.74	1.17	0.53	67.24	30.46
1.47	0.95	0.42	64.63	28.57
1.14	0.73	0.32	64.04	28.07
1.45	0.96	0.39	66.21	26.90
1.41	0.98	0.47	69.50	33.33
1.72	1.13	0.51	65.70	29.65
1.27	0.77	0.30	60.63	23.62
1.77	1.17	0.49	66.10	27.68
1.48	0.97	0.42	65.54	28.38
1.64	1.02	0.37	62.20	22.56
1.61	1.00	0.30	62.11	18.63
1.58	1.01	0.41	63.92	25.95
1.68	1.11	0.47	66.07	27.98
1.88	1.23	0.56	65.43	29.79
1.76	1.14	0.52	64.77	29.55
1.71	1.12	0.51	65.50	29.82
1.55	1.01	0.45	65.16	29.03
1.74	1.12	0.52	64.37	29.89
1.77	1.14	0.54	64.41	30.51
1.26	0.81	0.35	64.29	27.78
1.17	0.67	0.30	57.26	25.64
1.17	0.66	0.30	56.41	25.64
1.01	0.63	0.26	62.38	25.74
1.30	0.79	0.36	60.77	27.69
1.03	0.59	0.25	57.28	24.27
1.04	0.62	0.27	59.62	25.96
1.22	0.71	0.30	58.20	24.59
1.06	0.64	0.27	60.38	25.47

Table 5 4 Results of yields of Gulf sturgeons fed trout diet

Fish (Kg)	Bullet (Kg)	Dressed Fillet (Kg)	% Bullet	% Dressed Fillet
1 60	1 10	0 48	68 75	30 00
2.02	1 44	0 64	71 29	31 68
1 64	1 09	0 51	66 46	31 10
1 32	0 90	0 38	68 18	28 79
2.15	1 54	0 77	71 63	35 81
1 51	1 05	0 44	69 54	29 14
2 13	1 41	0 67	66.20	31 46
1 75	1 26	0 51	72 00	29 14
1 39	0 90	0 38	64 75	27 34
1 20	0 82	0 30	68.33	25 00
0 98	0 61	0 20	62.24	20 41
1 20	0 85	0 35	70 83	29 17
1 40	not determined	0 41	not determined	29 29
1 58	1 04	0 44	65 82	27 85
1 92	1 31	0 54	68 23	28 13
1 10	0 68	0 31	61 82	28 18
1 88	1 24	0 51	65 96	27 13
2 45	1 66	0 77	67 76	31 43
1 88	1 25	0 62	66 49	32 98
1.38	0 84	0 39	60 87	28 26
1 30	0 81	0 38	62 31	29 23
1 79	1 16	0 56	64 80	31 28
1 89	1 28	0 60	67 72	31 75
1 06	0 65	0 26	61 32	24 53
2 10	1 33	0 59	63 33	28 10
1 72	1 11	0 47	64 53	27 33
1 54	1 03	0 46	66 88	29 87
1 45	0 95	0 43	65 52	29 66
1 17	0 71	0 31	60 68	26 50
2.25	1.22	0 59	54 22	26 22

Table 5-5 presents the means and standard deviations (SD) for all treatments. It can be noticed that Gulf sturgeons fed catfish diet did not grow as much as those fed bass diet Mean weight of Gulf sturgeons fed trout diet was bigger than fish from the other groups

Table 5-5 Means and standard deviation of results of yields of Gulf sturgeon fed three different commercial diets

Treatment	Whole Fish (Kg)	Bullet (Kg)	Dressed Fillet (Kg)	% Bullet	% Dressed Fillet
Mean Catfish	1 21	0 74	0 33	61 01	27 42
SD Catfish	0 20	0 13	0 07	3 16	3 06
Mean Bass	1 47	0 94	0 41	63 43	27 54
SD Bass	0 27	0.21	0 10	3 30	3 06
Mean Trout	1 63	1 08	0 48	65 81	28 89
SD Trout	0 39	0.27	0 14	3 93	2 86

Analysis of variance was performed for fish weight, weights of dressed fillet, % bullet and % dressed fillet Results from analysis of variance of weights of whole fish and weights of dressed fillets are shown in Table 5-6 There is a significant difference between weights of whole fish fed different diets, as well as for the dressed fillets. Duncan's multiple range test was performed on data from whole fish and dressed fillets and the results are shown in Table 5-7 and Table 5-8 respectively There are significant differences between all groups for both variables

Table 5-6 Results of analysis of variance of fish weight and dressed fillets weights of Gulf sturgeons fed three different commercial diets

Source of Variation	SS Effect	DF Effect	MS Effect	SS Error	DF Error	MS Error	F-Value	p-Value
Whole Fish	2.691	2	1 345	7 602	87	0 087	15 406	1 87E-06
Dressed Fillets	0.303	2	0 151	1 031	87	0 011	12 814	1 33E-05

SS = sum of squares
DF = degrees of freedom
MS = Mean squares

Table 5-7 Results of Duncan's multiple range test of fish weight of Gulf sturgeons fed three different commercial diets (p-values)

Treatment	Trout	Bass	Catfish
Trout		0.043	5.38E-05
Bass	0.043		0.001
Catfish	5.38E-05	0.001	

Table 5-8 Results of Duncan's multiple range test of dressed fillet weights from Gulf sturgeons fed three different commercial diets (p-values)

Treatment	Trout	Bass	Catfish
Trout		0.015	5.61E-05
Bass	0.015		0.011
Catfish	5.61E-05	0.011	

Results of analysis of variance of % bullet and % dressed fillet are presented in Table 5-9. There are significant differences between groups with regard to % bullet, but there are no significant differences between groups with regard to % dressed fillet. It was observed earlier (Chapter 4) that there is a tendency of yield to increase with increase of fish size. Nonetheless, looking at the mean values for the fish weight from all treatments it can be noticed that there is a difference of less than 0.5 Kg between groups. In addition, bigger losses in terms of yields do not occur when preparing the bullets, but they do occur while trimming step to yield the dressed fillets. Therefore it is expected to find no differences in % of dressed fillets even though the weights of fish fed trout diet were bigger than the other groups. Note that the lack of differences in dressed fillet yields reflect the necessity of more trimming on fish fed trout due to larger amount of dark muscle under the skin.

Table 5-9 Results of analysis of variance for % bullet and % dressed fillet of Gulf sturgeons fed three different commercial diets

Source of Variation	SS Effect	DF Effect	MS Effect	SS Error	DF Error	MS Error	F-Value	p-Value
% Bullet	339 705	2	169 852	1038 565	86	12 076	14 064	5 19E-06
% Dressed Fillets	40 026	2	20 013	780 057	87	8 966	2 232	0 113

SS = sum of squares
DF = degrees of freedom
MS = Mean squares

Table 5-10 Results of Duncan's multiple range test for % bullet of Gulf sturgeon fed three different commercial diets (p-values)

Treatment	Trout	Bass	Catfish
Trout		0 010	5 44E-05
Bass	0 010		0 008
Catfish	5 44E-05	0 008	

Sensory evaluation

Results from triangle sensory panel show that a significant difference at a p-value of 0 01 was found between trout diet and bass diet. Most panelists rated the magnitude of the difference as slight Looking at the composition of trout diet it can be said that it is more similar to the composition of bass diet than to the catfish diet. Nonetheless, the results from the triangle test did not show difference between fillets of Gulf sturgeons fed catfish diet and fillets of Gulf sturgeons fed trout diet. Another triangle test should have been carried out to confirm these results. It does not seem reasonable that the consumer would be able to find differences between fish fed bass diet and fish fed trout diet, but not between fish fed catfish diet and fish fed trout diet This statement is based on the fact that bass diet is much more similar in composition to trout diet than trout diet is to catfish diet. However, little work has been done in the area of

fish diet and it effects on taste of cooked muscle, and composition of the diet itself is not the only factor to affect the quality taste of fish muscles.

The mean values and standard deviations (SD) of the mean of the scores from the consumer sensory panel for specific attributes of the steamed fillets are show in Table 5-11

Table 5-11 Mean and standard deviations of the scores from sensory evaluation of cooked sturgeon fillets fed three different commercial diets (p-values)

Treatment	Moisture	Tenderness	Fishiness	Rubbery	Likability
Mean Catfish	9 7	9 4	8 8	9 1	8 5
SD Catfish	2 8	2 7	4 0	3 2	3 0
Mean Bass	9 3	9 5	8 6	9 1	7 8
SD Bass	2 9	3 0	3 5	2 9	2 9
Mean Trout	7 7	8 9	7 9	8 5	7 8
SD Trout	3 4	2 9	3 9	3 5	3 0

Analysis of variance was performed for each attribute The results of analysis of variance for the attribute moisture are shown in Table 5-12. In this case there are no significant differences between panelists, but there is a significant difference between the treatments Thus Duncan's multiple range test was performed to point out which treatments were different, and results of this test are show in Table 5-13

Table 5-12 Results of analysis of variance for the attribute moisture from consumer sensory panel for steamed fillets of Gulf sturgeons fed three different commercial diets

Source of Variation	SS	DF	MS	F-Value	p-Value	F-Critical
Panelists	734 043	66	11 121	1 328	0 084	1 406
Treatments	154 516	2	77 258	9 231	0 0001	3 064
Error	1104 724	132	8 369			
Total	1993 283	200				

SS = sum of squares
DF = degrees of freedom
MS = Mean squares

The p-values shown in Table 5-13 indicate that moisture of steamed fillets of Gulf sturgeons fed catfish diet is not significantly different then steamed fillets of Gulf sturgeons fed bass diet. Additionally it can be seen moisture of steamed fillets of Gulf sturgeons fed trout diet are significantly different than steamed fillets of Gulf sturgeons fed bass and catfish diet

Table 5-13. Results of Duncan's multiple range test for the attribute moisture from consumer seusory panel for steamed fillets of Gulf sturgeons fed three different commercial diets (p values)

Treatment	Trout	Bass	Catfish
Trout		0.002	0.0001
Bass	0.002		0.429
Catfish	0.0001	0.429	

The results of analysis of variance for the attribute tenderness are shown in Table 5-14. In this case there was no significant difference between treatments but there was a significant difference between panelists. Differences between panelists are expected because there was no previous training of the panelists. Observe that the intention of this study was to access the possible differences found by fish consumers

Table 5-14. Results of analysis of variance for the attribute teuderness from consumer sensory panel for steamed fillets of Gulf sturgeons fed three different commercial diets

Source of Variation	SS	DF	MS	F-Value	p-Value	F-Critical
Panelists	862.474	66	13.067	2.239	4.44E-05	1.406
Treatments	16.106	2	8.053	1.379	0.255	3.064
Error	770.340	132	5.8359			
Total	1648.922	200				

SS = sum of squares
DF = degrees of freedom
MS = Mean squares

The results of analysis of variance for the attribute fishiness are shown in Table 5-15. In this case there was no significant differences between treatments but there was a significant difference between panelists

Table 5-15. Results of analysis of variance for the attribute fishiness from consumer sensory panel for steamed fillets of Gulf sturgeons fed three different commercial diets

Source of Variation	SS	DF	MS	F Value	p-Value	F-Critical
Panelists	1591.099	66	24.107	2.447	6.6E-06	1.406
Treatments	29.2781	2	14.639	1.485	0.230	3.064
Error	1300.435	132	9.851			
Total	2920.812	200				

SS = sum of squares
DF = degrees of freedom
MS = Mean squares

The results of analysis of variance for the attribute rubbery are shown in Table 5-16. In this case there were no significant differences between treatments but there was a significant difference between panelists

Table 5-16. Results of analysis of variance for the attribute rubbery from consumer sensory panel for steamed fillets of Gulf sturgeons fed three different commercial diets

Source of Variation	SS	DF	MS	F Value	p-Value	F-Critical
Panelists	1028.522	66	15.583	2.078	0.0002	1.406
Treatments	21.018	2	10.509	1.401	0.249	3.064
Error	989.622	132	7.497			
Total	2039.162	200				

SS = sum of squares
DF = degrees of freedom
MS = Mean squares

The results of analysis of variance for the attribute likability are shown in Table 5-17. In this case there were no significant differences between treatments but there was a significant difference between panelists

Table 5-17 Results of analysis of variance for the attribute likability from consumer sensory panel for steamed fillets of Gulf sturgeons fed three different commercial diets

Source of Variation	SS	DF	MS	F Value	p-Value	F-Critical
Panelists	797.140	66	12.077	1.648	0.008	1.406
Treatments	22.890	2	11.445	1.562	0.213	3.064
Error	967.056	132	7.326			
Total	1787.088	200				

SS = sum of squares
DF = degrees of freedom
MS = Mean squares

It can be seen from the results of this sensory study that the differences between treatments are very small. The catfish group received higher scores for likability, moisture and tenderness which are positive attributes, but at the same time it also received higher scores for fishiness and rubbery which are detrimental attributes in the quality of fish fillets. It can be concluded from these results that consumers do not perceive significant differences in the attributes studied between treatments. The mean values and standard deviations (SD) of the mean of the scores from the consumer sensory panel for specific attributes of the smoked fillets are show on Table 5-18.

Table 5-18 Mean and standard deviations of the scores form sensory evaluation of cooked sturgeon fillets fed three different commercial diets

Treatments	Moisture	Saltiness	Fattiness	Smoked	Likability
Mean Catfish	7.7	10.5	8.5	9.4	7.7
SD Catfish	3.1	2.8	3.1	2.5	3.1
Mean Bass	9.3	9.7	8.3	8.7	8.4
SD Bass	2.3	2.8	2.7	3.1	2.9
Mean Trout	9.4	8.8	7.8	8.5	9.3
SD Trout	2.8	2.8	3.2	2.6	3.4

Analysis of variance was performed for each attribute. The results of analysis of variance for the attribute moisture are shown in Table 5-19. In this case, there are

significant differences between panelists and also there is a significant difference between the treatments. Thus, Duncan's multiple range test was performed to point out which treatments were different, and results of this test are show in Table 5-20. The p-values shown in Table 5-20 indicate that moisture content of smoked fillets of Gulf sturgeon fed catfish diet is not significantly different then smoked fillets of Gulf sturgeon fed bass diet. Additionally, it can be seen moisture scores of smoked fillets of Gulf sturgeon fed trout diet are significantly different than smoked fillets of Gulf sturgeon fed catfish diet but not significantly different than scores for smoked fillets of Gulf sturgeon fed bass diet. This result was also observed for the steamed fillets.

Table 5-19. Results of analysis of variance for the attribute moisture from consumer sensory panel for smoked fillets of Gulf sturgeons fed three different commercial diets

Source of Variation	SS	DF	MS	F-Value	p-Value	F-Critical
Panelists	599.335	44	13.621	2.991	6.23E-06	1.514
Treatments	79.801	2	39.900	8.762	0.0003	3.100
Error	400.704	88	4.553			
Total	1079.842	134				

SS = sum of squares
DF = degrees of freedom
MS = Mean squares

Table 5-20. Results of Duncan's multiple range test for the attribute moisture from consumer sensory panel for smoked fillets of Gulf sturgeons fed three different commercial diets (p-values)

Treatment	Trout	Bass	Catfish
Trout		0.032	0.018
Bass	0.032		0.024
Catfish	0.018	0.024	

The results of analysis of variance for the attribute saltiness are shown in Table 5-21. In this case there was a significant differences between treatments and also between

panelists Results for Duncan's multiple range test are show in Table 5-22 The p values shown in Table 5-22 indicate that saltiness of smoked fillets of Gulf sturgeon fed catfish diet is not significantly different then smoked fillets of Gulf sturgeon fed bass diet. Additionally, it can be seen that saltiness of smoked fillets of Gulf sturgeon fed trout diet are significantly different than smoked fillets of Gulf sturgeon fed catfish diet but not significantly different than smoked fillets of Gulf sturgeon fed bass diet This difference is probably due to the fact that fish fed trout diet were larger than fish fed catfish diet Therefore, the fillets from fish belonging to the trout group were much thicker than fillets from fish belonging to the catfish group Note that the brining time was the same for all treatments, thus thinner fillets might have absorbed a proportionally higher amount of salt during brining than thicker fillets

Table 5-21 Results of analysis of variance for the attribute saltiness from consumer sensory panel for smoked fillets of Gulf sturgeon fed three different commercial diets

Source of Variation	SS	DF	MS	F-Value	p-Value	F Critical
Panelists	548 625	44	12 468	2 300	0 0004	1 514
Treatments	68 607	2	34 303	6 329	0 002	3 100
Error	476 892	88	5 419			
Total	1094 126	134				

SS = sum of squares
DF = degrees of freedom
MS = Mean squares

Table 5-22. Results of Duncan s multiple range test for the attribute saltiness from consumer sensory panel for smoked fillets of Gulf sturgeon fed three different commercial diets (p-values)

Treatment	Trout	Bass	Catfish
Trout		0 109	0 004
Bass	0 109		0 171
Catfish	0 004	0 171	

The results of analysis of variance for the attribute fattiness are shown in Table 5-23 In this case there was no significant differences between treatments but there was a significant difference between panelists Even though the most striking difference between the compositions of the diets is fat content, most fat is stored in the layer of dark muscle, which is thoroughly trimmed to yield dressed fillets This might explain the lack of differences found in the sensory analysis

Table 5-23 Results of analysis of variance for the attribute fattiness from consumer sensory panel for smoked fillets of Gulf sturgeon fed three different commercial diets

Source of Variation	SS	DF	MS	F Value	p Value	F-Critical
Panelists	816.245	44	18.551	4.007	1.53E-08	1.514
Treatments	12.865	2	6.432	1.389	0.254	3.100
Error	407.335	88	4.628			
Total	1236.445	134				

SS = sum of squares
DF = degrees of freedom
MS = Mean squares

The results of analysis of variance for the attribute smoked flavor intensity are shown in Table 5-24 In this case there was no significant differences between treatments but there was a significant difference between panelists

Table 5-24 Results of analysis of variance for the attribute smoked flavor intensity from consumer sensory panel for smoked fillets of Gulf sturgeon fed three different commercial diets

Source of Variation	SS	DF	MS	F Value	p-Value	F-Critical
Panelists	432.915	44	9.838	1.537	0.044	1.514
Treatments	22.914	2	11.457	1.790	0.172	3.100
Error	563.252	88	6.400			
Total	1019.082	134				

SS = sum of squares
DF = degrees of freedom
MS = Mean squares

The results of analysis of variance for the attribute likability are shown in Table 5-25. Significant differences between treatments and also between panelists were found. Duncan's multiple range test was performed to point out which treatments were different, and results of this test are show in Table 5-26. The p-values shown in Table 5-26 indicate that smoked fillets of Gulf sturgeon fed catfish diet is not significantly different then smoked fillets of Gulf sturgeon fed bass diet. Additionally, smoked fillets of Gulf sturgeon fed trout diet are significantly different than fillets of sturgeon fed catfish diet but not significantly different than smoked fillets of Gulf sturgeon fed bass diet. In this case consumers show a preference for fillets of Gulf sturgeon fed bass and trout diet. Fish fed trout diet will achieve commercial size faster, thus if the product is to be commercialized as smoked fillets, trout diet would be the best choice for the fish grower.

Table 5-25. Results of analysis of variance for the attribute likability from consumer sensory panel for smoked fillets of Gulf sturgeon fed three different commercial diets

Source of Variation	SS	DF	MS	F-Value	p-Value	F-Critical
Panelists	812.288	44	18.461	3.288	1.03E-06	1.514
Treatments	58.521	2	29.260	5.211	0.007	3.100
Error	494.085	88	5.6146			
Total	1364.895	134				

SS = sum of squares
DF = degrees of freedom
MS = Mean squares

Table 5-26. Results of Duncan s multiple range test for the attribute likability flavor intensity from consumer sensory panel for smoked fillets of Gulf sturgeon fed three different commercial diets (p-values)

Treatment	Trout	Bass	Catfish
Trout		0.163361	0.020629
Bass	0.163361		0.303644
Catfish	0.020629	0.303644	

The mean values and standard deviations (SD) from the scores of the appearance sensory test are presented on Table 5-27. As mentioned earlier the scores were replicated for each panelist. It ca be seen that the scores were quite different between fillets from fish fed trout diet and fillets from fish fed the other two diets. Analysis of variance was performed on the data collected during this study, and results show that there are no significant differences between replicates, but there are differences between treatments (Table 5-28). Results from Duncan's multiple range test show that all treatments are significantly different from each other (Table 5-29).

Table 5-27 Mean and standard deviations of the scores from consumer test of appearance of raw sturgeon fillets fed three different commercial diets

	Catfish	Catfish	Bass	Bass	Trout	Trout
Mean	7.5	7.3	6.9	6.3	3.9	3.9
SD	1.4	1.5	1.8	1.9	2.0	2.1

Table 5-28 Results of analysis of variance for consumer test for appearance of raw fillets of Gulf sturgeon fed three different commercial diets

Source of Variation	DF Effect	MS Effect	DF Error	MS Error	F-Value	p-Value
Replicated (a)	1	5.192	384	3.165	1.640	0.201
Treatment (b)	2	435.976	384	3.165	137.722	0.00001
Interaction (a x b)	2	3.838	384	3.165	1.212	0.298

SS = sum of squares
DF = degrees of freedom
MS = Mean squares

Table 5-29 Results of Duncan's multiple range test for consumer test for appearance of raw fillets of Gulf sturgeon fed three different commercial diets (p-values)

Treatment	{1}	{2}	{3}	{4}	{5}	{6}
Catfish group {1}		4.05E-06	0.084	0.693	4.29E-06	0.0002
Trout group {2}	4.05E-06		2.56E-06	4.29E-06	0.882	1.09E-05

Bass group {3}	0.084	2.56E-06		0.152	1.09E-05	0.048
Catfish group replicate {4}	0.693	4.29E-06	0.152		2.56E-06	0.0009
Trout group replicate {5}	4.29E-06	0.882	1.09E-05	2.56E-06		8.73E-06
Bass group replicate {6}	0.0003	1.09E-05	0.048	0.0009	8.73E-06	

Replicates were consistent in the study. The mean score from the likability scale for trout treatment (mean score = 3.9) indicates that consumers dislike slightly the appearance of these fillets. In addition, consumers like moderately fillets belonging to bass treatment (mean score = 6.6), and like very much fillets from catfish treatment (mean score = 7.4). As it can be seen on the ballot used form this test (Figure A-1), consumers were asked whether they would purchase any of the samples under investigation, the results are shown below:

- 89.4% of the panelist would purchase fillets of Gulf sturgeon
- 10.6% of the panelists would not purchase fillets of Gulf sturgeon
- 45.9 % of the panelists would just purchase fillets from the catfish group
- 18.7% of the panelists would just purchase fillets from the bass group
- 1.7% of the panelists would just purchase fillets from the trout group
- 28.9% of the panelists would purchase fillets from either catfish or bass group
- 5.1% of the panelists would purchase fillets from all groups

The results above are very encouraging in case aquaculture of Gulf sturgeon for profitable purposes is approved in the state of Florida. There is a strong possibility consumers would be willing to include Gulf sturgeon in their diet.

Proximate Analysis

The results of proximate analysis of the diets are shown in Table 3-30. Table 5-31 presents the results from the proximate analysis of raw fillets of Gulf sturgeon fed

trout, bass or catfish diet. The mean values and standard deviations from the mean (SD) are shown in Table 5-32. These results were subjected to analysis of variance. As it can be seen on Table 5-33, moisture content and ash content are significantly different between the treatments. Duncan's multiple range test was performed on data from moisture and ash content, and results are presented in Table 5-34 and Table 5-35.

Table 5-30. Proximate composition of commercial diets fed to Gulf sturgeon

Diets	Replicates	% Moisture	% Ash	% Fat	% Protein
Trout Diet	1	12.99	5.63	13.14	53.52
Trout Diet	2	12.82	5.40	13.82	51.69
Bass Diet	1	13.08	7.27	6.69	49.81
Bass Diet	2	13.13	7.00	6.01	50.73
Catfish Diet	1	12.67	9.21	1.32	40.48
Catfish Diet	2	12.70	9.09	1.58	42.62

Table 5-31. Proximate composition of raw fillets of Gulf sturgeon fed three different commercial diets

Treatment	Replicates	% Moisture	% Ash	% Fat	% Protein
Trout Fish 1	1	79.99	0.88	0.95	17.30
Trout Fish 1	2	80.11	0.91	1.07	17.15
Trout Fish 2	1	79.90	0.93	1.08	16.91
Trout Fish 2	2	79.86	0.95	1.13	17.04
Trout Fish 3	1	79.04	0.92	1.50	17.43
Trout Fish 3	2	78.87	0.94	1.22	17.52
Bass Fish 1	1	80.02	0.83	1.08	18.12

Table 5 33 continued

Treatment	Replicates	% Moisture	% Ash	% Fat	% Protein
Bass Fish 1	2	79 91	0 80	1 28	17 88
Bass Fish 2	1	80 37	0 78	1 37	17 93
Bass Fish 2	2	80 44	0 79	1 12	16 94
Bass Fish 3	1	80 69	0 79	0 93	18 03
Bass Fish 3	2	80 82	0 81	0 98	17 37
Catfish Fish 1	1	81 48	0 74	0 98	17.35
Catfish Fish 1	2	81 86	0 76	0 94	17 62
Catfish Fish 2	1	81 65	0 78	1 03	16 98
Catfish Fish 2	2	81 17	0 74	1 09	17 52
Catfish Fish 3	1	81 44	0 82	0 96	17 09
Catfish Fish 3	2	81 13	0 79	1 03	18 01

Table 5-32 Means and standard deviation of raw fillets of Gulf sturgeon fed three different commercial diets

Treatment	% Moisture		% Ash		% Fat		% Protein	
	Means	SD	Means	SD	Means	SD	Means	SD
Catfish	81 49	0.32	0 77	0 03	1 01	0 06	17 56	0 38
Bass	80 38	0.36	0 80	0 02	1 13	0 17	17 71	0 46
Trout	79.63	0.53	0 92	0 03	1 16	0 19	17 23	0 24

Table 5-33 Results of analysis of variance from proximate composition of raw fillets of Gulf sturgeon fed three different commercial diets

Source of Variation	SS Effect	DF Effect	MS Effect	SS Error	DF Error	MS Error	F-Value	p-Value
% Moisture	10 513	2	5 256	2 556	15	0 170	30 844	4 84E-06
% Ash	0 076	2	0 038	0 009	15	0 0006	59 747	7 17E-08
% Fat	0 078	2	0 039	0 339	15	0 022	1 738	0 209332
% Protein	0 746	2	0 373	2 033	15	0 135	2 753	0 095819

SS = sum of squares
DF = degrees of freedom
MS = Mean squares

Table 5-34 Results of Duncan's multiple range test on moisture content of raw fillets of Gulf sturgeon fed three different commercial diets (p-values)

Treatmeot	Catfish	Bass	Trout
Catfish		0.0004	8.93E-05
Bass	0.0004		0.0069
Trout	8.93E-05	0.0069	

Table 5-35 Results of Duncan's multiple range test on ash content of raw fillets of Gulf sturgeon fed three different commercial diets (p-values)

Treatment	Catfish	Bass	Trout
Catfish		0.0711	8.88E-05
Bass	0.0711		0.0001
Trout	8.88E-05	0.0001	

Even though the main difference between the diets is fat content, there is no significant difference in fat content of raw fillets from the different treatments. According to Ackman's (1990) classification of lipid content in fish, fillets of Gulf sturgeon are considered lean for all treatments. It was observed during filleting that fish belonging to the trout group presented a thicker layer of dark muscle than fish belonging to the catfish group. Therefore, it seems that Gulf sturgeon stores most of the fat in the dark muscle layer which was thoroughly trimmed during filleting. This explains the lack of difference in fat content found between the treatments. There are no significant differences in protein content between the treatments. There is a significant difference in moisture content between all treatments. Fillets belonging to the catfish group present higher moisture content than fillets from the bass group. Fillets belonging to the trout group present lowest moisture content. Nonetheless these differences are smaller than 2%. Note that ash content in trout group is significantly different than ash content of catfish and bass group. However, this is due to the fact that standard deviations from the means are very small for all groups studied.

Table 5-36 presents the results from the proximate analysis of smoked fillets of Gulf sturgeon fed trout, bass or catfish diet. The mean values and standard deviations from the mean (SD) are shown in Table 5-37. The results were subjected to analysis of variance. As it can be seen in Table 3-38, ash content is significantly different between the treatments. Duncan's multiple range test was performed on data from ash content, and results are presented in Table 5-39.

Table 5-36 Proximate composition of smoked fillets of Gulf sturgeon fed three different commercial diets

Treatment	Replicates	% Moisture	% Ash	% Fat	% Protein
Trout Fish 1	1	70.14	4.09	3.53	22.33
Trout Fish 1	2	70.10	4.12	3.33	22.59
Trout Fish 2	1	69.83	4.19	3.80	22.21
Trout Fish 2	2	70.00	4.20	3.47	22.38
Trout Fish 3	1	70.01	4.13	3.93	21.96
Trout Fish 3	2	69.95	4.16	3.73	22.05
Bass Fish 1	1	69.42	4.11	4.47	22.10
Bass Fish 1	2	69.30	4.06	4.13	21.78
Bass Fish 2	1	69.72	4.05	3.53	22.65
Bass Fish 2	2	69.67	4.07	3.77	22.47
Bass Fish 3	1	70.01	4.09	4.00	21.82
Bass Fish 3	2	70.08	4.13	3.87	22.04
Catfish Fish 1	1	70.37	3.77	3.00	22.73
Catfish Fish 1	2	70.28	3.87	2.93	22.95
Catfish Fish 2	1	69.60	3.98	4.13	22.30
Catfish Fish 2	2	69.65	4.09	4.33	22.44
Catfish Fish 3	1	70.47	4.13	3.60	21.87
Catfish Fish 3	2	70.39	4.02	3.87	22.06

Table 5-37 Means and standard deviation of smoked fillets of Gulf sturgeon fed three different commercial diets

Treatment	% Moisture		% Ash		% Fat		% Protein	
	Means	SD	Means	SD	Means	SD	Means	SD
Catfish	70 01	0 11	4 15	0.043	3 63	0 23	22.25	0.23
Bass	69 70	0 31	4.09	0.031	3.96	0 32	22.14	0 35
Trout	70 13	0 39	3.98	0.14	3.64	0 58	22.39	0 41

Table 5-38 Results of analysis of variance from proximate composition of raw fillets of Gulf sturgeon fed three different commercial diets

Source of Variation	SS Effect	DF Effect	MS Effect	SS Error	DF Error	MS Error	F-Value	p-Value
% Moisture	0 579	2	0 289	1 315	15	0.087	3 304	0 064
% Ash	0.090	2	0 045	0.106	15	0.007	6 388	0.009
% Fat	0.420	2	0 210	2 458	15	0.163	1 283	0 305
% Protein	0 185	2	0.092	1 696	15	0.113	0 821	0 458

SS = sum of squares
DF = degrees of freedom
MS = Mean squares

Table 5 39 Results of Duncan's multiple range test on ash content of smoked fillets of Gulf sturgeon fed three different commercial diets (p-values)

Treatment	Catfish	Bass	Trout
Catfish		0.212	0 004
Bass	0.212		0.041
Trout	0 004	0.041	

There are no significant differences in protein and lipid content between the smoked fillets from the different treatments, and this is in agreement with data from raw fillets. According to Ackman's (1990) classification of fat content for fish, smoked fillets of Gulf sturgeon are considered low fat for all treatments Note that there is a loss of 10% water during drying and smoking processes. Ash content of fillets from trout group is slightly different than fillets from catfish and bass group. Observe than standard

deviations from the mean of % ash from fillets belonging to bass and catfish group are very small The overall difference in ash content between the groups is 0 2% The increase in ash content can be related to the salt uptake during brining Salt uptake for fillets of Gulf sturgeon fed catfish diet was 3 48%, for fillets of Gulf sturgeon fed bass diet was 3.29% and for fillets of Gulf sturgeon fed trout diet was 3 07%. These results support the sensory scores for saltiness, where catfish group received a score of 10 5, bass group a score of 9.7 and trout group 8 8 Panelists were able to discriminate catfish group from trout group with regard to salt content and correctly assign catfish group as the group presenting higher salt content Notice that the difference is about 0 4 % in salt content between the groups Once again, it should be pointed out that the differences in the thickness of the fillets cause differences in the salt content of the product, if fillets are brined for the same amount of time, in brines with identical salt concentration It can be seen from results that more care should have been taken to avoid different salt contents between treatments Fillets from catfish group should have been brined for a shorter length of time or in a brine solution of lower concentration to reach the same salt content as trout group

Color Analysis

Based on sensory score for appearance test, there is a difference in appearance between the treatments. Figure 5 3 shows the center slices of fillets from the Gulf sturgeon fed catfish diet, bass diet and trout diet There is a markedly difference in the color between treatments Fillets of Gulf sturgeon fed trout diet present a yellowish orange coloration, while fillets from fish fed Catfish diet and bass diet present pale pink coloration This difference might explain why trout preformed so poorly on the consumer sensory test for appearance of the raw fillets Figure 5-4 shows center slices of

fillets from bass treatment over time It can be seen in the picture below that fish fed trout diet tend to develop a yellow-orange color, not present on the fillets from fish from the other two diet treatments

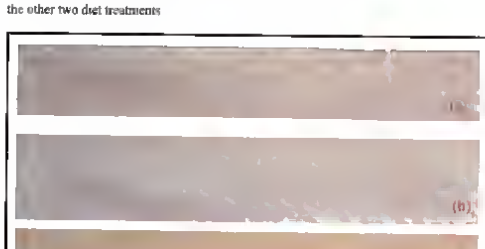

Figure 5-3 Center slices of Gulf sturgeons fed three different commercial diets (a) bass treatment; (b) catfish treatment, (c) trout treatment

A color study over time was carried out to quantify these differences, and monitor color changes over four different storage times during 15 days of storage Lab values were measured at days 0, 5, 10 and 15 using CR-200 Chroma Meter and machine vision It is important to mention that these Lab values cannot be readily compared because the instruments use different sources of light and equations must be used to convert values to the same source (Luzuriaga, 1999) Machine vision uses a 5000^{0}K fluorescent light (illuminant D50), which simulates color qualities of noonday summer sun (Luzuriaga, 1999) Colorimeter CR200 can use illuminant C, which represents average daylight with

a color temperature of 6774°K, or illuminant D65 which is the average daylight with a color temperature of 6504 °K. In this study illuminant D65 was used as the source of light. Results from the different equipments will be presented separately and statistical analysis performed on the data collected from each instrument independently. Results form statistical analysis will be compared and conclusions presented with regard to performance of instruments.

Machine Vision System

Machine vision collects one picture from each fillet at every interval of time. The picture is calibrated using the standard tile and then Lab values calculated for each picture. The frames were grabbed with Color Analysis software version 2.6.9 (author Diego Luzuriaga). The calibration step and Lab calculation is done with Color Analysis NFG version 2.6.8 (author Diego Luzuriaga). Lab values were determined for whole fillets and for a center slice of the fillets. The pictures of the center slices of the fillets of Gulf sturgeon fed catfish diet are shown in Figure C-1 (Appendix C). The pictures of the center slices of fillets of Gulf sturgeon fed bass diet are shown in Figure C-2 (Appendix C). The pictures of the center slices of fillets of Gulf sturgeon fed trout diet are shown in Figure C-3 (Appendix C).

It was noticed that the non uniform thickness of Gulf sturgeon fillets influenced the results of Lab values calculated. This is due to the fact that the edges of a fillet are thinner than the center and have some translucence. Thus, the color of the background interferes with the actual color of the fillet. This causes a problem during the calibration step. Calibration is done by first calculating the Lab value for the color of the tile as it appears in the frame. Then, calculating the difference between these values with the

standard Lab value provided by the tiles manufacturer. Finally, every pixel in the frame is adjusted according to the differences in Lab values found for the tile. Figure C-4 shows the orange tile and three fillets (one for each treatment) before and after calibration (Appendix C). The color description for the calibrated picture of the catfish fillet shown in Figure C-4 is pale yellow green which is different from grayish yellowish pink which is the description of the center slices of the fillets (Figure C-1). The color description for the calibrated picture of the bass fillet shown in Figure C-4 is pale yellow green which is different from grayish yellowish pink which is the description of the center slice of the fillet (Figure C-2). The color description for the calibrated picture of the trout fillet shown in Figure C-4 is grayish yellow, which is different from moderate yellowish pink which is the description of the center slice of the fillet (Figure C-3). This discrepancy in color description is going to be reflected on the Lab values assigned for each frame, as it can be seen in Tables C-1 and in Table C-2. The a values are especially affected: observe that on the center slices a values are slightly positive, while on the whole fillets the a values are slightly negative. This shift reflects the green tones that appear after calibration.

The mean L values and standard deviations (SD) for the whole fillets are shown in Table 5-40. The mean a values and mean b values together with the standard deviations of the means for whole fillets are shown in Table 5-41 and Table 5-42 respectively.

Table 5-40 Mean L values and standard deviations of whole fillets of Gulf sturgeons fed three different commercial diets

Treatment	Day 0	Day 5	Day 10	Day 15
Means Catfish	79.50	79 72	80 37	81 19
SD Catfish	1 49	1 16	0 84	1.15
Means Bass	80 77	80.08	81 19	81.87
SD Bass	0.74	0 52	0.87	0.57
Means Trout	77 52	77.43	79.03	80 20
SD Trout	1 33	0 96	1.42	1.08

Table 5-41 Mean a values and standard deviations of whole fillets of Gulf sturgeons fed three different commercial diets

Treatment	Day 0	Day 5	Day 10	Day 15
Means Catfish	-4 33	5 85	-4 60	-5.46
SD Catfish	1.51	0 71	0 8	1.58
Means Bass	5 43	-5.46	-4.67	5 73
SD Bass	0.94	0 96	1.31	0.80
Means Trout	1 94	-3.25	-2 87	3 50
SD Trout	2.60	1.28	2 22	1 05

Table 5-42 Mean b values and standard deviations of whole fillets of Gulf sturgeons fed three different commercial diets

Treatment	Day 0	Day 5	Day 10	Day 15
Means Catfish	13 12	14 3	13 81	13 83
SD Catfish	1 05	1.79	2.85	0 63
Means Bass	12 86	15 28	12 91	14.40
SD Bass	2 09	1 54	3.41	1 81
Means Trout	23.44	26.80	26 01	25.23
SD Trout	10.20	9.90	8 93	8 81

Figure 5-4 is a plot of mean Lab values from machine vision for treatments using the whole fillets of Gulf sturgeon fed three different commercial diets Statistical analysis was not performed on this data because of the discrepancy in the color of the samples after calibration.

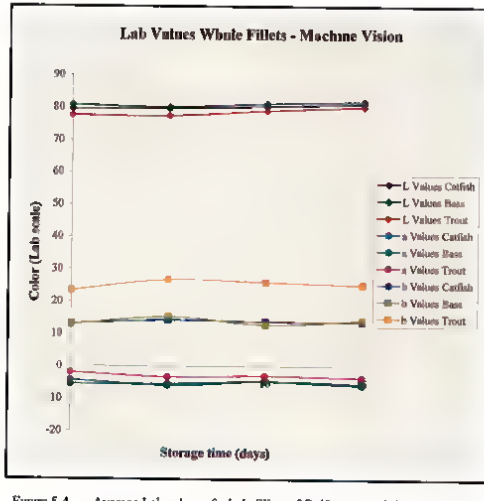

Figure 5-4. Average Lab values of whole fillets of Gulf sturgeon fed three different commercial diets measured by machine vision

Figure C-5, Figure C-6 and Figure C-7 in Appendix C show the plots of Lab values determined by machine vision for each fish from catfish, bass and trout treatment respectively. Statistical analysis was performed on the results for the center slices of the fillets. Even though this is a repetitive measurement design with multiple fish in each treatment, the statistical analysis showed that the repetitive measurements correlation structure was not significant. Therefore, the variables tested in the model were time, treatment and the interaction between time and treatment. The model used can be described as a mixed linear model with random effect for fish within a treatment. The covariance parameter estimates from the statistical analysis of L values are presented in Table 5-43. Table 5-44 shows that treatment, days and the interaction between treatment and days are significantly different for L values at a p-level of 0.05.

Table 5-43. Covariance parameter estimates of L values for center slices of fillets from Gulf sturgeons fed three different commercial diets.

Covariance Parameter	Ratio	Estimate	Standard Error	Z Value	p-Value
Fish (treatment)	2.153	0.603	0.275	2.19	0.014
Residual	1.000	0.280	0.066	4.24	0.0001

Table 5-44. Statistical results of L values for center slices of fillets from Gulf sturgeon fed three different commercial diets.

Effect	DF Effect	DF Error	F-Value	p-Value
Treatment (t)	2	12	7.85	0.006
Days (d)	3	36	85.44	0.0001
Interaction (d x t)	6	36	2.81	0.023

DF = degrees of freedom

Mean comparisons for L values were done using Least Squares Difference test (LSD) using Bonferroni adjustment. This adjustment makes the LSD test more conservative (Rao, 1998). Table 5-45 presents the results for differences between days and differences between treatments. The overall result for treatment shows that L values of fillets from bass treatment are significantly different than fillets from trout group. L values of fillets from catfish treatment are not significantly different than the L values of the other two treatments. The overall results for days show that L values are significantly different for all intervals of time.

The mean L values and standard deviations of the means together with the results of LSD test for the interaction between days and treatment are shown in Table 5-46. Significant differences within a day for the different treatments are expressed with superscript letters. Significant differences within a treatment for the different days of storage are expressed as subscript letters. Treatments with same letters are not significantly different at a p-level of 0.05. Fillets from all treatments present color fading over time. However on catfish treatment L values do not vary significantly from day 5 up to 15 days of storage. L values of fillets from bass treatment on day 0 are significantly different from all intervals of time but day 5, day 5 is just significantly different than day 15 and day 10 is significantly different than day 0. L values of trout treatment are significantly different for all intervals of time. During the study L values of fillets from catfish treatment were not significantly different than the ones from bass treatment for all intervals of time. L values from trout treatment were significantly different than L values form the other treatments just up to day 5. By day 10 L values for all treatments converged and were not significantly different up to the end of the study. It is noticeable

from the results that trout treatment shows more fading than other treatments. The magnitude of the change in L values between day 0 and day 15 of fillets from bass treatment is 2 61 and for catfish treatment is 2 69, but because the fillets from bass treatment show smaller standard deviations than catfish treatment, the statistical test is able to separate the intervals of time within the treatment It can be said that the changes in L values from day 0 to day 15 for bass treatment and catfish treatment are very similar about 3.5% and 3 4% respectively Trout treatment presents a change of 5 1% in L value between day 0 and day 15

Table 5-45 Differences of least square means using Bonferroni adjustment of L values for center slices of fillets from Gulf sturgeons fed three different commercial diets

Effect	Treatment	Days	Treatment	Days	p-Value
Treatment	Bass		Catfish		0 3200
Treatment	Bass		Trout		0 0058
Treatment	Catfish		Trout		0 1422
Days		0		5	0 0001
Days		0		10	0 0001
Days		0		15	0 0001
Days		5		10	0 0054
Days		5		15	0 0001
Days		10		15	0 0001

Table 5-46 Mean L values and standard deviations of center slices of fillets from Gulf sturgeons fed three different commercial diets

Treatment	Day 0	Day 5	Day 10	Day 15
Means Catfish	76 81 $^{xy}_{a}$	78 42 $^{xy}_{b}$	78 51 $^{x}_{b}$	79 50 $^{x}_{b}$
SD Catfish	1 21	1 28	0 66	0 74
Means Bass	77 79 $^{x}_{c}$	78 90 $^{x}_{cd}$	79 64 $^{x}_{de}$	80 40 $^{x}_{e}$

Table 5-46 continued

Treatment	Day 0	Day 5	Day 10	Day 15
SD Bass	0 88	0 49	0 83	0 74
Means Trout	75 26 $^{y}{}_{f}$	76 50 $^{y}{}_{g}$	77 78 $^{x}{}_{h}$	79 11 $^{x}{}_{i}$
SD Trout	1 13	0 84	1.27	0 83

Superscript letters indicate significant different between treatments within a day at p=0 05
Subscript letters indicate significant difference between days within a treatment at p=0.05

The covariance parameter estimates from the statistical analysis of a values are presented in Table 5-47 Table 5-48 shows that treatment, days and the interaction between treatment and days are significantly different for a values at a p-level of 0 05

Table 5-47 Covariance parameter estimates of a values for center slices of fillets from Gulf sturgeons fed three different commercial diets

Covariance Parameter	Ratio	Estimate	Standard Error	Z Value	p-Value
Fish (treatment)	2 3149	2.02085	1 0006	2.21	0 0136
Residual	1 0000	0 9540	0.2249	4.24	0 0001

Table 5-48 Statistical results for a values of center slices of fillets from Gulf sturgeon fed three different commercial diets

Effect	DF Effect	DF Error	F-Value	p-Value
Treatment (t)	2	12	6 17	0 0144
Days (d)	3	36	11.01	0.0001
Interaction (d x t)	6	36	2 66	0 0308

DF = degrees of freedom

Mean comparisons for a values were done using Least Squares Difference test (LSD) using Bonferroni adjustment. Table 5-49 presents the results for differences between days and differences between treatments The overall results for treatment show

that a values of fillets from catfish treatment are not significantly different than bass treatment. Trout treatment is significantly different than the two other treatments. The overall results for days show that day 0 and day 10 are significantly different than day 15, but day 5 is not significantly different than day 15. In addition, day 0 is not significantly different than day 10 or day 5, but day 5 and day 10 are significantly different.

The mean a values and standard deviations of the means together with the results of LSD test for the interaction between days and treatment are shown in Table 5-50. Significant differences within a day for the different treatments are expressed with superscript letters. Significant differences within a treatment for the different days of storage are expressed as subscript letters. Treatments with same letters are not significantly different at a p-level of 0.05. On day 0 the mean a value of fillets from trout treatment is about 30% higher than fillets from catfish treatment, and about 40% higher than fillets from bass treatment. Nonetheless, there is no significant difference on day 0 between fish from catfish group and trout group. The standard deviation of a values on trout treatment is large and explain the overlap in values with catfish treatment. Significant differences were not found in any other interval of time for any treatments. Thus, even though trout treatment presents higher intensity of color red at day 0, changes with storage bring the a value to levels similar of the one found on the other treatments by day 5. Differences within catfish treatment were not found over storage for a values. Results for bass and catfish treatment show that at day 10 a values increased about 35% compared to day 0, and than at day 15 the values were about the same as day 0. There is no explanation for this pattern of change. The a values of fillets from trout diet treatment tend to decrease over time and there is a significant difference between day 0 and day 15

Table 5-49 Differences of least square means using Bonferroni adjustment of a values for center slices of fillets from Gulf sturgeons fed three different commercial diets

Effect	Treatment	Days	Treatment	Days	p-Value
Treatment	Bass		Catfish		1 0000
Treatment	Bass		Trout		0 0355
Treatment	Catfish		Trout		0 0268
Days		0		5	0 2552
Days		0		10	0 4121
Days		0		15	0 0085
Days		5		10	0 0019
Days		5		15	1 0000
Days		10		15	0 0001

Table 5 50 Mean a values and standard deviations of the center slices of fillets of Gulf sturgeons fed three different commercial diets (machine vision)

Treatment	Day 0	Day 5	Day 10	Day 15
Means Catfish	7.85 $^{xy}_{a}$	6 47 $^{z}_{a}$	8 06 $^{x}_{a}$	6.53 $^{z}_{a}$
SD Catfish	1 69	1 12	1.35	1 64
Means Bass	6 65 $^{x}_{b}$	7.06 $^{x}_{bc}$	9 05 $^{x}_{c}$	6.74 $^{y}_{b}$
SD Bass	0 41	0 90	1 72	0 80
Means Trout	11 40 $^{y}_{d}$	10 11 $^{x}_{dc}$	10 79 $^{x}_{dc}$	8 93 $^{x}_{c}$
SD Trout	2.94	2 16	2 84	1.82

Superscript letters indicate significant different between treatments within a day at p=0 05
Subscript letters indicate significant difference between days within a treatment at p=0 05

The covariance parameter estimates from the statistical analysis of b values are presented in Table 5-51 Table 5-52 shows that treatment and days are significantly different for b values but the interaction between days and treatment is not significantly different

Table 5-51 Covariance parameter estimates for b values of the center slices of fillets from Gulf sturgeons fed three different commercial diets

Covariance Parameter	Ratio	Estimate	Standard Error	Z Value	p-Value
Fish (treatment)	12 3151	23 6589	9.8554	2.40	0 0082
Residual	1 0000	1 9211	0 4528	4 24	0 0001

Table 5-52 Statistical results for b values of center slices of fillets of Gulf sturgeon fed three different commercial diets

Effect	DF Effect	DF Error	F-Value	p-Value
Treatment (t)	2	12	7 33	0 0083
Days (d)	3	36	15 52	0 0001
Interaction (d x t)	6	36	1 71	0 1471

DF degrees of freedom

Mean comparisons for b values were done using Least Squares Difference test (LSD) using Bonferroni adjustment. Table 5-53 presents the results for differences between days and differences between treatments. The overall results of b values between treatments is the same found for a values, where trout is significantly different than the other treatments, but catfish treatment is not significantly different than bass treatment The overall results for days show that day 0 is significantly different than all other intervals of time. Day 5 is also significantly different than all other intervals of time On the other hand, day 10 and day 15 are not significantly different from each other

The mean b values and standard deviations of the means together with the results of LSD test for the interaction between days and treatment are shown in Table 5 54 Significant differences within a day for the different treatments are expressed with

superscript letters. Significant differences within a treatment for the different days of storage are expressed as subscript letters. Treatments with same letters are not significantly different at a p-level of 0.05. The standard deviation of b values for trout treatment is very large and because of that was not possible to show significant differences between groups. Nonetheless, b values on day 0 are twice as big for trout treatment when compared to values from bass and catfish treatment. The plot for b values of the center slices of the fillets shown in Figure C-7 (Appendix C) show that Fish Q and M present b values are within the range of fish from other treatments, but Fish P, M and R present much higher values. This difference is also evident in Figure C-3 (Appendix C). Trout diet imparts in some cases a yellow-orange coloration to the fillets of Gulf sturgeon. Results from the sensory panel for appearance show that this color is unappealing to consumers.

It is noteworthy to mention that Nickellan and Bromage (1998) have shown that increasing dietary lipids in rainbow trout increased the efficiency of deposition of the colorant astaxathin (carophyll pink). The researchers also found out that deposition of this colorant was much higher at the tail region of the rainbow trout fillet, when compared to dorsal and belly areas. Therefore, pigmentation of the fillets was not a uniform process (Nickellan and Bromage, 1998). The possible source of colorant on the trout diet is unknown, but it can be pointed out that in case of Gulf of Mexico sturgeon fillets, the development of yellow-orange tones in some fish fed trout diet is a quality problem.

Table 5-53 Differences of least square means using Bonferroni adjustment of b values for center slices of fillets from Gulf sturgeons fed three different commercial diets

Effect	Treatment	Days	Treatment	Days	p-Value
Treatment	Bass		Catfish		1.0000
Treatment	Bass		Trout		0.0169
Treatment	Catfish		Trout		0.0203
Days		0		5	0.0001
Days		0		10	0.0056
Days		0		15	0.0061
Days		5		10	0.0167
Days		5		15	0.0153
Days		10		15	1.0000

Table 5-54 Mean b values and standard deviations of the center slices of fillets from Gulf sturgeons fed three different commercial diets

Treatment	Day 0	Day 5	Day 10	Day 15
Means Catfish	$10.97^{x}{}_{a}$	$13.23^{x}{}_{a}$	$12.53^{xy}{}_{a}$	$12.16^{x}{}_{a}$
SD Catfish	0.94	1.04	2.67	0.96
Means Bass	$10.11^{x}{}_{b}$	$14.04^{x}{}_{c}$	$10.90^{y}{}_{bc}$	$12.64^{x}{}_{bc}$
SD Bass	2.19	1.26	2.68	1.46
Means Trout	$20.12^{x}{}_{d}$	$24.29^{x}{}_{d}$	$23.26^{x}{}_{d}$	$21.83^{x}{}_{d}$
SD Trout	9.14	8.62	8.27	7.42

Superscript letters indicate significant different between treatments within a day at $p<0.05$
Subscript letters indicate significant difference between days within a treatment at $p<0.05$

CR-200 Chroma Meter

CR-200 is a hand-held colorimeter. An average of three spots per fillet were collected at every interval of time. Lab values for each fillet at any given interval of time is an average of the three readings. Table C-3 shows the values of the three readings for each fillet from the catfish treatment together with the mean values and standard

deviations from the means (Appendix C) Table C-4 shows the values of the three readings for each fillet from the bass treatment together with the mean values and standard deviations from the means (Appendix C) Table C-5 shows the values of the three readings for each fillet from the trout treatment together with the mean values and standard deviations from the means (Appendix C)

The covariance parameter estimates from the statistical analysis of L values are presented in Table 5-55 Table 5-56 shows that treatment, days and the interaction between treatment and days are significantly different for L values

Table 5-55 Covariance parameter estimates of L values for fillets from Gulf sturgeons fed three different commercial diets

Covariance Parameter	Ratio	Estimate	Standard Error	Z Value	p-Value
Fish (treatment)	0.4739	1.0367	0.6592	1.57	0.0579
Residual	1.0000	2.1875	0.5156	4.24	0.0001

Table 5-56 Statistical results of L values for fillets from Gulf sturgeon fed three different commercial diets

Effect	DF Effect	DF Error	F-Value	p-Value
Treatment (t)	2	12	6.06	0.0152
Days (d)	3	36	41.24	0.0001
Interaction (d x t)	6	36	222.39	0.0479

DF = degrees of freedom

Mean comparisons for L values were done using Least Squares Difference test (LSD) using Bonferroni adjustment. Table 5-57 presents the results for differences between days and differences between treatments. Overall results of L values from Chroma Meter show that fillets from catfish treatment are not significantly different than

the one from the other two treatments. In addition, fillets from trout treatment are significantly different than fillet from bass treatment. This result is in accordance with results found for the center slices of fillets determined by machine vision. The overall results of L values for days show that day 0 is significantly different than all other intervals of time. Day 5 is also significantly different than all other intervals of time. Day 10 and day 15 are not significantly different from each other.

The mean L values and standard deviations of the means together with the results of LSD test for the interaction between days and treatment are shown in Table 5-58. Significant differences within a day for the different treatments are expressed with superscript letters. Significant differences within a treatment for the different days of storage are expressed as subscript letters. Treatments with same letters are not significantly different at a p-level of 0.05. There are no changes in L value for catfish treatment over time. L values within bass and trout treatment behave the same way, and day 0 is significantly different than days 5, 10 and 15. However, days 5, 10 and 15 are not significantly different from each other. At Day 10 a significant difference between trout and bass treatment can be noticed, but there are no other significant differences between treatments at any given interval of time. Results from CR-200 Chroma meter also show that some fading occurs on fillets from trout and bass diet over time.

Table 5-57. Differences of least square means using Bonferroni adjustment of L values for fillets from Gulf sturgeons fed three different commercial diets

Effect	Treatment	Days	Treatment	Days	p-Value
Treatment	Bass		Catfish		1.0000
Treatment	Bass		Trout		0.0195
Treatment	Catfish		Trout		0.0649

Table 5-57 continued

Effect	Treatment	Days	Treatment	Days	p-Value
Days		0		5	0 0001
Days		0		10	0 0001
Days		0		15	0.0001
Days		5		10	0 0022
Days		5		15	0 0007
Days		10		15	1 0000

Table 5-58 Mean L values and standard deviations of fillets from Gulf sturgeons fed three different commercial diets

Treatment	Day 0	Day 5	Day 10	Day 15
Means Catfish	60 2 $^{x}_{a}$	61 1 $^{x}_{a}$	62 6 $^{xy}_{a}$	63.5 $^{x}_{a}$
SD Catfish	2.8	2 4	1 4	1 5
Means Bass	58 2 $^{x}_{b}$	62.0 $^{x}_{c}$	64 7 $^{y}_{c}$	64 5 $^{x}_{c}$
SD Bass	1 7	1 1	1 4	0 7
Means Trout	55.5 $^{x}_{d}$	59 7 $^{x}_{e}$	61 9 $^{x}_{e}$	61 7 $^{x}_{e}$
SD Trout	1 1	2 2	1 5	2 9

Superscript letters, indicate significant different between treatments within a day at p=0.05
Subscript letters, indicate significant difference between days within a treatment at p=0.05

The covariance parameter estimates from the statistical analysis of a values are presented in Table 5-59 Table 5-60 shows that days and the interaction between treatment and days are significantly different for a values, but there is no significant difference between treatments

Table 5-59. Covariance parameter estimates of a values for fillets from Gulf sturgeons fed three different commercial diets

Covariance Parameter	Ratio	Estimate	Standard Error	Z Value	p-Value
Fish (treatment)	2.2105	0.3238	0.1474	2.20	0.0140
Residual	1.0000	0.1465	0.03452	4.24	0.0001

Table 5-60. Statistical results of a values for fillets from Gulf sturgeon fed three different commercial diets

Effect	DF Effect	DF Error	F-Value	p-Value
Treatment (t)	2	12	0.88	0.4406
Days (d)	3	36	4051.42	0.0001
Interaction (d x t)	6	36	2.8	0.0242

DF = degrees of freedom

Mean comparisons of a values was done using Least Squares Difference test (LSD) using Bonferroni adjustment. Table 5-61 presents the results for differences between days and differences between treatments. The a values are not significantly different for treatments but there are significant differences between days. The a values found for day 0 and day 10 are not significantly different from each other but both are significantly different than a values for days 5 and 15. In addition, a values for day 5 and day 15 are not significantly different form each other.

The mean a values and standard deviations of the means together with the results of LSD test for the interaction between days and treatment are shown on Table 5-62. Significant differences within a day for the different treatments are expressed with superscript letters. Significant differences within a treatment for the different days of storage are expressed as subscript letters. Treatments with same letters are not

significantly different at a p-level of 0.05. The a values of day 0 are significantly different from the a values of days 5, 10 and 15 for all the three treatments. Moreover, days 5, 10 and 15 are not significantly different. Results show that the a values for the different treatments are not significantly different on days 0, 5, and 15. On day 10 a values of bass treatment are significantly different than trout treatment. Note that the a values on day 0 are positive for all treatment, but drop to values around −1 by the fifth day of storage. After day 5 a values drop very little. Thus, results indicate that there is a large fading of red pigments that occur from day 0 to day 5, which is not consistent with results determined by machine vision.

Table 5-61. Differences of least square means using Bonferroni adjustment of a values for fillets from Gulf sturgeons fed three different commercial diets.

Effect	Treatment	Days	Treatment	Days	p-Value
Treatment	Bass		Catfish		0.8710
Treatment	Bass		Trout		1.0000
Treatment	Catfish		Trout		0.7766
Days		0		5	0.0001
Days		0		10	0.0001
Days		0		15	0.0001
Days		5		10	1.0000
Days		5		15	0.0018
Days		10		15	0.0003

Table 5-62. Mean a values and standard deviations of fillets form Gulf sturgeons fed three different commercial diets.

Treatment	Day 0	Day 5	Day 10	Day 15
Means Catfish	$12.6^{x}{}_{a}$	$-1.1^{x}{}_{b}$	$-1.4^{x}{}_{b}$	$-1.8^{x}{}_{b}$
SD Catfish	0.5	0.5	0.4	0.7

Table 5-62 continued

Treatment	Day 0	Day 5	Day 10	Day 15
Means Bass	10.8 $^{x}_{c}$	-1 6 $^{x}_{d}$	1 3 $^{x}_{d}$	2.2 $^{x}_{d}$
SD Bass	0.3	0.3	0 6	0 4
Means Trout	10 4 $^{x}_{f}$	-1 5 $^{x}_{g}$	-1 3 $^{x}_{g}$	-2 1 $^{x}_{g}$
SD Trout	1.5	0 7	0 9	0 9

Superscript letters: indicate significant different between treatments within a day at p=0.05
Subscript letters: indicate significant difference between days within a treatment at p=0.05

The covariance parameter estimates from the statistical analysis of b values are presented in Table 5-63. Table 5-64 shows that treatment, days and the interaction between treatment and days are significantly different for L values.

Table 5-63 Covariance parameter estimates of b values for fillets from Gulf sturgeons fed three different commercial diets.

Covariance Parameter	Ratio	Estimate	Standard Error	Z Value	p-Value
Fish (treatment)	3 0302	1 7928	0 7930	2 26	0.0119
Residual	1 0000	0.5916	0 1395	4 24	0 0001

Table 5-64 Statistical results of b values for fillets from Gulf sturgeon fed three different commercial diets

Effect	DF Effect	DF Error	F-Value	p-Value
Treatment (t)	2	12	11 58	0.0016
Days (d)	3	36	1408 48	0.0001
Interaction (d x t)	6	36	2 31	0 0550

DF = degrees of freedom

Mean comparisons for b values were done using Least Squares Difference test (LSD) using Bonferroni adjustment. Table 5-65 presents the results for differences

between days and differences between treatments Overall results from b values are similar to the ones for a values, where trout treatment is significantly different than catfish and bass, but the two last ones are not significantly different from each other The overall results for days show that day 0 is significantly different than days 5, 10, and 15 Moreover day 10 are not significantly different from day 5 but it is significantly different than day 15

The mean b values and standard deviations of the means together with the results of LSD test for the interaction between days and treatment are shown in Table 5-66 Significant differences within a day for the different treatments are expressed with superscript letters Significant differences within a treatment for the different days of storage are expressed as subscript letters Treatments with same letters are not significantly different at a p-level of 0 05 The results for b values show that the only significant difference between treatments occurs on day 15 where trout treatment is significantly different than catfish treatment Within treatments, day 0 is significantly different than the other intervals of time Day 5, 10 and 15 are not significantly different from each other for any of the treatments. This result follows the same pattern of results found for a values for the individual treatments over storage The results from machine vision also indicate 'yellowing' of the fillets over time, but the pattern of the change in b values is very different Figure 5-5 is a plot of the mean Lab values for each treatment for data determined by machine vision using the center slices of the fillets Figure 5-6 is a plot of the mean Lab values for each treatment for data determined by CR-200 Chroma Meter using three measurements for each fillets Note that as mentioned above, the

pattern of the changes of a and b values are very different for the different instruments

On the other hand, L values follow a similar pattern of change for both instruments

Table 5-65 Differences of least square means using Bonferroni adjustment of b values for fillets from Gulf sturgeons fed three different commercial diets

Effect	Treatment	Days	Treatment	Days	p-Values
Treatment	Bass		Catfish		1.0000
Treatment	Bass		Trout		0.0057
Treatment	Catfish		Trout		0.0028
Days		0		5	0.0001
Days		0		10	0.0001
Days		0		15	0.0001
Days		5		10	0.6915
Days		5		15	0.0300
Days		10		15	1.0000

Table 5-66 Mean b values and standard deviations of fillets from Gulf sturgeons fed three different commercial diets

Treatment	Day 0	Day 5	Day 10	Day 15
Means Catfish	-14.6 $^{x}{}_{a}$	-0.1 $^{x}{}_{b}$	-0.1 $^{x}{}_{b}$	0.5 $^{x}{}_{b}$
SD Catfish	0.4	1.0	1.0	1.3
Means Bass	-15.1 $^{x}{}_{c}$	0.5 $^{x}{}_{d}$	0.6 $^{x}{}_{d}$	1.0 $^{xy}{}_{d}$
SD Bass	0.9	0.5	1.0	1.0
Means Trout	-5.7 $^{y}{}_{e}$	2.8 $^{x}{}_{g}$	4.1 $^{y}{}_{g}$	4.8 $^{y}{}_{g}$
SD Trout	2.0	2.1	3.1	2.1

Superscript letters: indicate significant different between treatments within a day at p=0.05
Subscript letters: indicate significant difference between days within a treatment at p=0.05

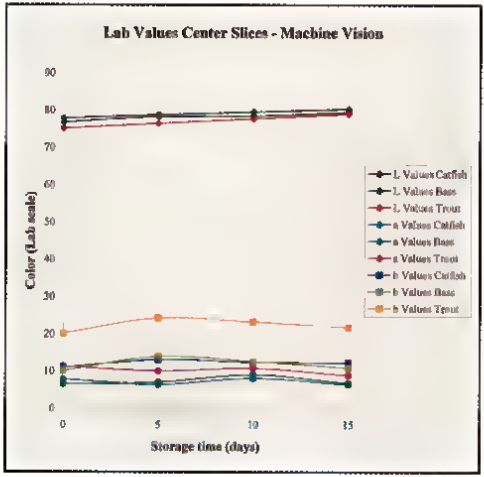

Figure 5-5 Average Lab values of center slices of fillets of Gulf sturgeon fed three different commercial diets measured by machine vision

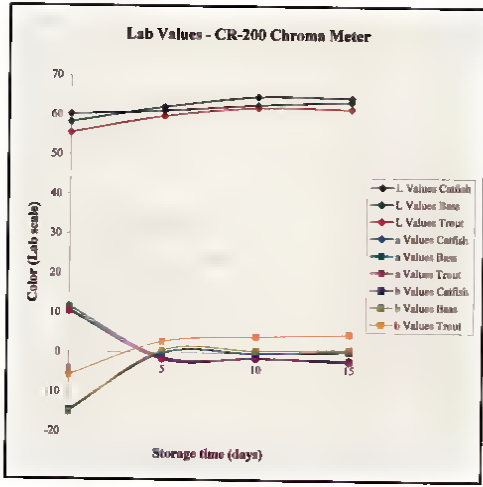

Figure 5-6 Average Lab values of fillets of Gulf sturgeon fed three different commercial diets measured by CR-200 Chroma Meter

Table 5-67 is a summary of all statistical results performed on data for both instruments. The results for effect of treatment from L and b values are the same for both instruments. However, for a values CR-200 shows no significant difference between treatments while machine vision shows a significant difference between trout and bass, where tout presented the highest a values of all treatments. The results for effect of storage days are different between machine vision and CR-200. The statistical modal separates the L values of all storage days as significantly different from each other on data from machine vision. The same model indicates from L values from CR-200 that day 10 is not significantly different than day 15. In addition, a values from machine vision show no significant difference between day 0 and day 5, while data from CR-200 show the opposite. Results from b values from machine vision show that day 10 and day 15 are not significantly different while CR-200 shows day 5 and day 10 as not significantly different. Results for the interaction of treatments within days of storage for L values from machine vision separates trout treatment from bass treatment on the first two intervals of time, while results from CR-200 shows significant difference between trout and bass just on day 10. Results for the interaction of treatment within days of storage for a values from machine vision show that the only significant difference is between trout and bass treatment on day 0, while CR-200 points out a significant difference between these two treatments just on day 10. Results for the interaction of treatment within days of storage for b values from machine vision show that the only significant difference is between trout and bass treatment on day 10. CR-200 shows significant differences on day 0 between catfish and bass treatment from trout treatment, and it also separates catfish from trout treatment on day 15. Finally, the interaction of

days of storage within a treatment show that for CR-200 there is a clear change in Lab values from day 0 to day 5 for all treatments, and then form that point on changes are not significant. Results from machine vision are very different in regard to the pattern of change of Lab values over time for the different treatments. It shows that L values of trout treatment increase significantly at every interval of time, while on catfish treatment the only significant change happens between day 0 and day 5. In addition, L values for bass treatment increase significantly between day 0 to day 10, and from day 5 to day 15. Trout treatment shows more fading than the other two treatments. In addition, it can be said that from machine vision results, a and b values do not vary within treatments considerably over 15 days of storage.

It is important to point out once again that trout group show very large standard deviation of b values. However, b values are still about twice as large for this group when compared with results from other treatments. Note that both instruments are able to pick up this difference especially on day 0. Three fish out of five from this group present a yellow-orange coloration, which is more pronounced on earlier times of storage. This can be considered a defect in quality that should be avoided in case commercialization of the fresh fillet is intended.

Table 5-67 Summary of the results of the statistical analysis on Lab values for fillets from Gulf sturgeon fed three different commercial diets determined by Machine Vision System and with CR-200 Chroma Meter

Instrument	Machine Vision			CR-200 Chroma Meter		
Effect	L	A	b	L	a	b
Treatment (T)	b c t	c b t	c b t	b c t	c b t	b c t
Days (D)	0 5 10 15	15 5 0 10	0 5 10 15	0 5 10 15	0 10 5 15	0 5 10 15
Day 0 x T	t c b	c b t	c b t	c b t	c b t	c b t
Day 5 x T	t c b	c b t	c b t	c b t	c b t	c b t
Day 10 x T	c b t	c b t	b c t	b c t	c b t	b c t
Day 15 x T	c b t	c b t	c b t	c b t	c b t	c b t
Catfish (c)x D	0 5 10 15	0 5 10 15	0 5 10 15	0 5 10 15	0 5 10 15	0 5 10 15
Bass (b)x D	0 5 10 15	15 0 5 10	0 10 15 5	0 5 10 15	0 5 10 15	0 5 10 15
Trout (t)x D	0 5 10 15	0 5 10 15	0 5 10 15	0 5 10 15	0 5 10 15	0 5 10 15

Fatty Acid Profile

The data from the chromatograms for all samples analyzed can be found on Appendix D. Figure D-1 and Figure D-2 present typical chromatograms of fatty acid methyl esters of Gulf sturgeon lipid. Peaks identified in this study are presented in different color for the different classes of fatty acids. Table 5-68 summarizes the results of fatty acid classes for the study as well as data reported by Chen et al. (1995). Note that the results from Appendix D were recalculated in order to disregard the percentage of unidentified peaks reported in the chromatograms. Thus, the sum of the concentration of saturated fatty acids, monoenes, dienes, trienes, tetraenes, pentaenes and hexaenes equals 100%. Results from a fatty acid study in Gulf sturgeon are also reported in Table 5-68 so data can be compared to fatty acid profile of fish found in the wild.

Table 5-68 Profile of fatty acid classes in feed and fillets of Gulf sturgeon fed different commercial diets

Sample	Saturated	Monoenes	Dienes	Trienes	Tetraenes	Pentaenes	Hexaene	ω 3	ω 6	ω 3 / ω 6
Catfish Diet	20.78	21.14	47.90	4.60	4.13	1.06	0.39	5.89	48.32	0.12
Raw Catfish	27.58	29.15	20.85	4.90	5.01	5.00	7.53	15.13	26.24	0.58
Smoked Catfish	28.52	26.25	19.58	4.47	5.51	5.87	9.76	17.26	26.74	0.65
Bass Diet	30.95	25.03	18.11	4.04	5.04	9.07	7.84	22.29	19.25	1.16
Raw Bass	28.42	31.16	15.28	3.44	4.65	7.48	9.58	20.16	17.78	1.13
Smoked Bass	29.26	27.28	12.84	2.83	4.62	10.09	13.10	25.36	16.26	1.56
Trout Diet	31.22	25.76	17.42	4.51	3.75	10.11	7.18	22.72	18.62	1.22
Raw Trout	29.14	27.57	14.52	3.23	4.36	9.42	11.73	24.81	16.83	1.47
Smoked Trout	29.77	27.53	14.33	3.27	4.18	9.35	11.59	24.43	16.70	1.46
Cultured Sturgeon [a]	26.80	40.50	4.41	1.09	4.04	10.90	12.20	24.30	7.02	3.58
Wild Sturgeon [b]	31.80	51.00	1.70	0.86	3.26	5.30	6.10	11.30	4.64	2.48

[a] fed commercial salmon diet (Chen et al., 1995)
[b] Chen et al. (1995)

The possible sources of lipids in ingredients list found in the packaging of the diets are presented below

- Catfish Diet – Soybean Meal, Fish Meal, Cottonseed Meal

- Hybrid Bass Diet – Soybean Meal, Fish Meal, Fish Oil
- Trout Diet - Soybean Meal, Fish Meal, Fish Oil

Soybean oil is on average mainly composed of the following fatty acids 11% palmitic (16 0), 4% stearic (18 0), 23 4% oleic (18 1ω9), 53 2% linoleic (18 2ω6) and 7 8% linolenic (18 3ω3) (White, 1992) Cottonseed oil is mainly composed of the following fatty acids 12.2 % palmitic 2 2% stearic 17 6 % oleic and 53 3% linoleic (White, 1992) Manhaden oil is mainly composed of the following fatty acids 7.3% myristic (14 0), 19% palmitic 9 1% palmitoleic (16 1ω7), 13 2% oleic, 1 3% linoleic, 1 35 linolenic 11% eicosapentaenoic (20 5ω3) and 9 1% docosahexaenoic (22 6ω3) (Ackman, 1989) Commercial herring oil is composed of 19 3 % of omega 3 fatty acids and 0.5 of omega-6 fatty acids thus the ratio ω 3 / ω 6 is about 39 3 (Piggot, 1989) The metabolic pathways of elongation of omega-6 and omega-3 fatty acids are distinct, and they compete for the same enzymes (Figure 5-7). Because the concentration of enzymes is finite increasing available of linoleic acid relative to concentration of linolenic acid will decrease the rate of synthesis of DHA In addition, arachidonic acid (AA) has been associated with platelets aggregation which leads to vessel constriction and higher risk of coronary heart disease (Lands, 1991) Thus high levels of AA can be counteractive to the health benefits of docosahexenoic acid (DHA) mentioned in Chapter 2

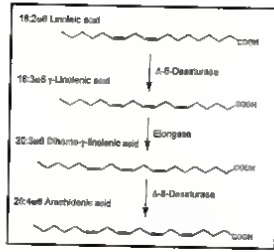

Figure 5-7 Omega-6 metabolic pathway Source O'Keefe (1998)

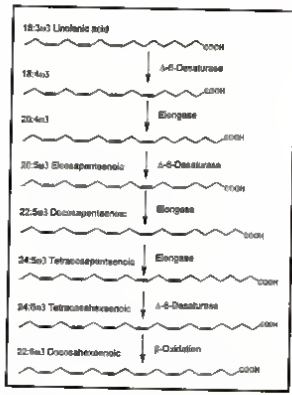

Figure 5-8 Omega 3 metabolic pathway Source: O'Keefe (1998)

Figure 5-9 is a graphic representation of data presented in Table 5-6B. The profile of fatty acid classes of trout diet is similar to hybrid bass diet. Wild sturgeon present about 50% monounsaturated fatty acids (MUFA), and none of the diets including salmonid diet (Chen et al. 1995) provide equivalent amounts of this class of fatty acids to the fish. Catfish diet provides the lowest amount of MUFA and large amount of dienes, which is mainly due to high levels of linoleic acid. Note that wild Gulf sturgeon contains just 1.7% of dienes, while sturgeon used during this study show levels between 14-20%, depending on the diet. There is no information regarding the proportion of the ingredients used in each diet, however the results show that Gulf sturgeon fed catfish diet contain very low levels of omega-3 fatty acids and high amounts of omega-6 fatty acids. The ratio ω3 / ω6 in fillets of sturgeon fed catfish diet are three times smaller than fish fed trout diet and about five times smaller than the ratio found for wild fish. This indicates that sturgeon fed the catfish diet have much lower relative amounts of omega-3 fatty acids, which could have negative nutrition consequences. Note that the salmonid diet fed to sturgeon in the study carried out by Chen et al. (1995) provided much lower level of dienes and a higher ratio of ω3 / ω6 fatty acids than catfish diet. This shows that it is possible to boost levels of omega-3 fatty acids in Gulf sturgeon, while keeping omega-6 fatty acids level low. A number of researchers suggest that aquaculture fish should be fed diets that maintain high omega-3 fatty acids in the muscle, so that the nutritional value is similar to wild caught fish. Smoking under the conditions used does not seem to present any adverse effect in the fatty acid composition of Gulf sturgeon fillets. Smoking affects mainly the chemical composition of surface of the fillet. The whole fillet is transformed into a paste to undergo fat extraction, so any changes in the

fatty acid profile that might have occurred at the surface of the fillet end up giving a small contribution to the overall fatty acid profile of the smoked sample.

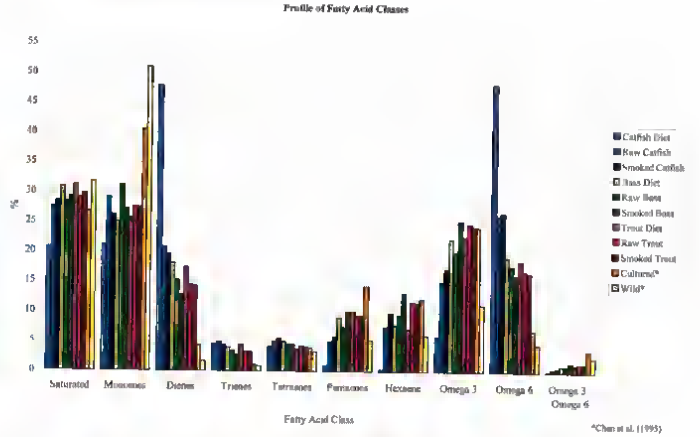

Figure 5-9 Profile of fatty acid classes on diets and Gulf sturgeon samples

Table 5-69 and Table 5-70 summarizes the results of fatty acids found in concentration equal or higher than 1%, as well as data from previous study (Chen et al., 1995). Note that the data found on Appendix D was recalculated in order to disregard the percentage of unidentified peaks reported in the chromatograms. Thus, the sum of the concentration of all fatty acids identified equals 100%. Figure 5-10 and 5-11 are graphic representations of results found in Table 5-69 and Table 5-70 respectively. The level of oleic acid in wild sturgeon is two times higher than that found in trout diet fed fish, and about a third higher than fish fed bass or catfish diet. On the other hand, levels of linoleic acid are about fifteen times higher in all diet groups, when compared to levels found in wild fish. It can be pointed out that linoleic acid is present in high levels in many oilseeds, and that the dietary levels of linoleate in human diet are 10 to 60 fold greater than the midpoint value estimated as ideal (Lands, 1990). Lands suggests that decreasing the source of omega 6 fatty acids and increasing the source of omega-3 fatty acids helps decreasing the risk of eicosanoid-related diseases. In a matter of fact, it can be seen in Table 5-70 that all metabolic products from 18:2ω6 are present in much higher amounts in fillets of sturgeon fed catfish diet. The level of arachidonic acid (AA) in this treatment is two times higher than that found in wild sturgeon. Bass diet provides the lowest levels of AA. The level of long chain highly unsaturated fatty acids were not adversely affected by diet. Trout diet provides the largest amount of DHA in the meat.

Table 5-69 Saturated, monosaturated and disaturated fatty acids found at concentrations over 1% on diets and Gulf sturgeon samples

Sample	14:0	16:0	18:0	16:1n7	18:1n9	18:1n7	20:1n9	16:2n6	18:2n6
Catfish Diet	0.45	15.55	2.80	0.85	16.60	1.32	0.45	0.02	45.97
Raw Catfish	2.39	19.47	4.52	3.11	19.98	2.87	1.11	0.24	18.70
Smoked Catfish	1.62	18.83	6.47	2.11	18.73	2.74	1.16	0.18	17.33
Bass Diet	4.75	19.76	3.98	5.37	13.51	2.44	1.11	0.74	16.93
Raw Bass	3.26	20.32	3.39	4.67	20.00	3.15	1.13	0.44	13.76
Smoked Bass	2.46	19.60	5.60	3.45	17.83	3.20	0.89	0.32	11.48
Trout Diet	5.24	20.20	4.58	8.53	13.38	2.41	1.46	1.21	16.41
Raw Trout	3.31	19.74	4.42	4.41	16.67	3.05	1.04	0.55	13.17
Smoked Trout	3.41	20.50	4.13	4.44	16.83	2.99	1.39	0.62	12.84
Raw Cultured [a]	2.20	17.60	5.72	3.33	24.30	4.84	2.45	0.82	1.94
Raw Wild [b]	1.43	25.90	2.61	1.92	35.70	2.74	1.11	0.26	0.40

[a] Gulf sturgeon fed commercial salmon diet (Chen et al. 1995)
[b] Wild Gulf sturgeon (Chen et al 1995)

Table 5-70 Triene, tetraene, pentaene and hexaene fatty acids found at concentrations over 1% on diets and Gulf sturgeon samples

Sample	18:3n3	18:3n6	18:3n3	20:3n6	18:4n3	18:4n1	20:4n6	20:5n3	22:5n3	22:6n3
Catfish Diet	0.07	0.06	4.20	0.09	0.06	3.70	0.22	0.44	0.08	0.38
Raw Catfish	0.28	1.24	1.22	1.61	0.55	1.39	2.13	2.98	1.40	7.53
Smoked Catfish	0.21	1.04	0.98	1.06	0.35	0.75	3.57	3.66	1.61	9.76
Bass Diet	1.11	0.36	2.02	0.11	1.85	1.53	0.52	2.17	1.21	7.84
Raw Bass	0.40	0.62	1.29	0.52	0.53	1.65	1.12	4.00	2.02	9.58
Smoked Bass	0.35	0.48	0.95	0.52	0.40	1.03	2.07	7.21	2.28	13.10
Trout Diet	1.26	0.45	2.45	0.12	1.95	0.48	0.55	0.30	1.33	7.45
Raw Trout	0.54	0.45	1.52	0.17	0.81	0.74	1.52	6.79	1.98	11.73
Smoked Trout	0.51	0.48	1.47	0.26	0.81	0.80	1.35	6.85	1.82	11.59
Raw Cultured [a]	0.00	0.00	0.20	0.00	0.10	0.00	1.00	8.30	2.20	12.20
Raw Wild [b]	0.00	0.00	0.30	0.20	0.30	0.00	1.40	1.78	2.28	6.10

[a] Gulf sturgeon fed commercial salmon diet (Chen et al., 1995)
[b] Wild Gulf sturgeon (Chen et al 1995)

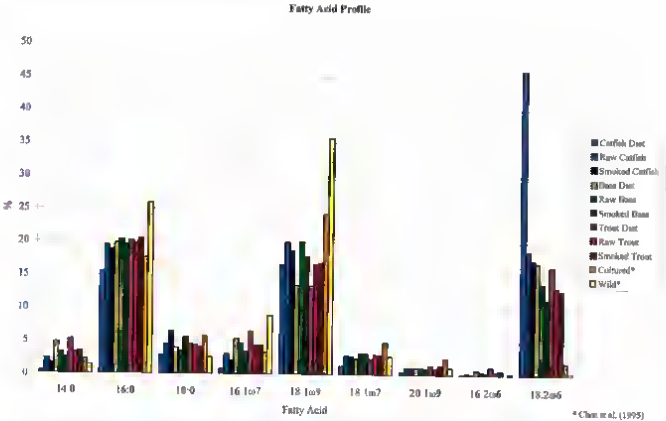

Figure 5-10 Profile of saturated fatty acid, monoenes and dienes on diets and Gulf sturgeon samples

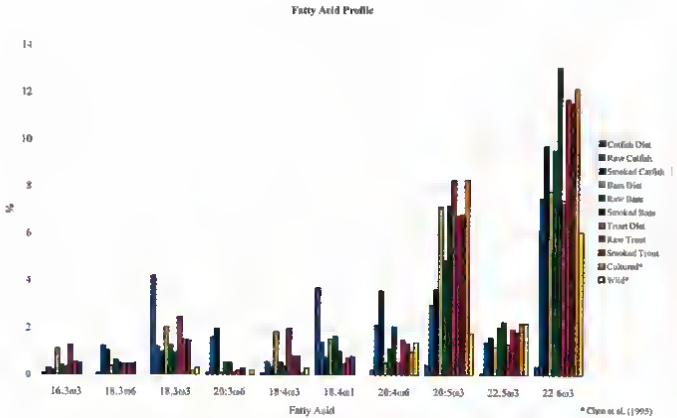

Figure 5-11 Profile of trienes, tetraenes, pentaenes and hexaene on diets and Gulf sturgeon samples

It can be concluded from this study that catfish diet is the least suitable of the diets tested in regard to nutritional benefits to human consumption. Feeding sturgeon with hybrid bass diet or trout diet results in fillets with similar fatty acid profiles. However, trout diet has higher level of DHA and higher ratio of ω3 / ω6 fatty acids, which are both positive aspects to human nutrition

Conclusions

A summary of the conclusions form each study is presented below in the same order the studies were described in this chapter

- There is a significant difference in weight of the whole fish among treatments Mean weights of Gulf sturgeon fed trout diet are the largest, followed by fish fed hybrid bass diet. Fish fed catfish diet presented the smallest mean weight.
- There is a significant difference in weight of the dressed fillets among treatments that follows the same patter as whole weight of the fish.
- There is a significant difference in fillet yields that follows the same pattern as whole weight of the fish
- There are no significant differences of yield of the dressed fillets This indicates that trimming of fillets of Gulf sturgeon fed trout diet had greater impact on decreasing yields than on other treatments One explanation might be that the layer of dark meat in sturgeon fed trout diet is larger than fish fed the other diets

- Triangle sensory panel indicated that there was a significant difference between cooked fillets of bass and trout treatment at p level of 0 01 The difference perceived by the panelist was rated as slight.
- Results of sensory panel for attributes of the cooked fillets showed no significant differences between the treatments for attributes of tenderness, rubbery, fishiness and likability Scores for moisture of fillets of sturgeon farm fish fed trout diet were significantly different than fillets of fish fed catfish diet. Moisture score for catfish treatment was 9 7, for bass treatment 9.3, and for trout treatment 7 7
- Results for sensory panel for attributes of the smoked fillets showed significant differences in the attributes moisture, saltiness and likability and no significant differences on attributes fattiness and smoked flavor intensity Moisture scores for trout treatment was 9 4, bass treatment 9 3 and catfish treatment 7 7 Note that this is the opposite of scores found for the cooked meat Saltiness of fillets fed catfish diet was considered significantly different than fillets of fish fed trout diet Fillets of fish fed trout diet are larger and thicker than fillets from fish fed catfish diet Therefore, the brining time or concentration of brining solution prior to smoking should have been adjusted to compensate for differences in sizes among treatments
- Consumer sensory panel to judge appearance of raw fillets show that consumers dislike slightly the appearance of fillets from trout treatment,

like moderately fillets from bass treatment, and like very much fillets from catfish treatment

- Results from color study show that fading of fillets from trout group is about 5% and about 3.5% for the other two treatments. The b values of trout treatment are twice as large as b values for the other treatments. Because the standard deviation of b values of trout fillets is so large, it was not possible to separate this group from the others. Some fillets from trout group present a yellow-orange coloration, this is detrimental to the quality of fish if it is to be commercialized fresh.
- Machine vision has limitations with regard to samples with non-uniform thickness, especially when the thinner areas are translucent. In this case, the color of the background interferes on the process of calibration leading to colors in the frame that are inexistent on the sample.
- CR-200 Chroma Meter shows patterns of change of Lab over time that differ from data determined by machine vision. Picture shown of Figure C-1 to C-3 on Appendix C do not show abrupt changes in color between day 0 and day 5 for any of the treatments as data from CR-200 suggests.
- The proximate analysis of the raw fillets from sturgeon fed the different diets is very similar. There are significant differences found in moisture and ash content, but the standard deviations are so small for the groups that mean values are very close together to be accounted as different.
- The proximate analysis of the smoked fillets showed that about 10 % of moisture is loss through smoking at the chosen conditions. Ash content

was significantly different among the groups. Salt uptake was highest in catfish and lowest in trout

- Fatty acid analysis indicated that catfish provides a poor fatty acid profile to Gulf sturgeon fillets. The ratio of ω3 / ω6 is most favorable in fish fed trout diet. Nonetheless, hybrid bass diet provided similar fatty acid profile to the muscle as it does trout diet. Smoking of sturgeon fillets does not seem to affect adversely the fatty acid profiles of the muscle

CHAPTER 6
CONCLUSIONS

This research provided information on methods of handling Gulf of Mexico sturgeon after removal of fish from culturing tanks up to commercialization the process of rigor mortis in the species, and influence of feeds on quality of the muscle

The findings of this study demonstrated that it is possible to monitor rigor mortis in Gulf sturgeon using a novel video capture methodology However more research needs to be conducted in order to determine its applicability to other fish species The influence of the shape of the fish body in relation to capability of the system to correctly assign frames in rigor and out of rigor also needs to be evaluated In addition, it was possible to determine rigor onset and resolution in Gulf sturgeon In the event this species is ever approved for commercialization, this information will help to ensure the quality of the product.

Processing yields for dressed fillet were determined and correlated to fish size There was a tendency for yields to increase with increase in fish weight. The variability on the proximate composition within fillets was investigated, and it was found that there were very small differences in the composition between parts of the fillet Thus, Gulf sturgeon dressed fillets do not show considerable variability in composition within a fillet This information will be useful in future studies, because it shows that it is possible to run repeated measurement experiments sampling within fillets

Results from the study on the influence of diet type in the quality of Gulf sturgeon fillets showed that trout diet provided the highest ratio of ω3 / ω6 fatty acids in the fillets However, results from color analysis and consumer sensory test for appearance revealed that feeding Gulf sturgeon with trout diet imparts an odd yellowish-orange coloration to the fillets which was unappealing to consumers Fillets of sturgeons fed catfish diet had a low ratio of ω3 / ω6 fatty acids In addition, the fatty acid profile of fish fed catfish diet was markedly different from the profile of wild sturgeon muscle.. Hybrid bass diet was suggested as the most adequate commercial diet to feed cultured sturgeons This conclusion was based on the fact that fillets from sturgeon fed bass diet performed well on sensory test, it did not present odd color pigmentation as trout diet, and imparts a fatty acid profile similar to the one determined for fillets from fish fed trout diet. It was suggested that Gulf sturgeon accumulates most of its fat in the sub-dermal layer of dark muscle, which was removed during trimming to yield the dressed fillets studied.

Finally there were very small differences in the proximate composition of the fillets from sturgeons fed different diets A possible explanation to the lack of differences in fat content among fillets is that Gulf sturgeon accumulates most of its fat in the sub-dermal layer of dark muscle Because all the dark muscle was removed during trimming to yield the dressed fillets studied, the differences in fat content among the diets was not reflected in the fat content of the dressed fillets

APPENDIX A
BALLOTS FOR SENSORY TESTS

Evaluation of Appearance of Gulf Sturgeon Raw Fillets

Name: _____ Date / /

Panelist # ____

Instructions

1. You have been presented with three trays of sturgeon fillets. Pretend that you are at the grocery store and evaluate the samples for overall appearance. Inspect the trays in the order presented below and indicate with a check mark, how much you like or dislike each group of Fillets

Tray #	Tray #	Tray #
____ like extremely	____ like extremely	____ like extremely
____ like very much	____ like very much	____ like very much
____ like moderately	____ like moderately	____ like moderately
____ like slightly	____ like slightly	____ like slightly
____ neither like nor dislike	____ neither like nor dislike	____ neither like nor dislike
____ dislike slightly	____ dislike slightly	____ dislike slightly
____ moderately dislike	____ moderately dislike	____ moderately dislike
____ very much dislike	____ very much dislike	____ very much dislike
____ dislike extremely	____ dislike extremely	____ dislike extremely

3. Would you purchase any of the samples on a given tray?

____ YES
____ NO

4. If the answer of the question above is YES, please write the tray(s) number below:

5. Comments.

_____

Thank you!

Figure A-1 Ballot of appearance sensory test (affective sensory test)

TRIANGLE SENSORY PANEL FOR COOKED STURGEON SAMPLES

Date / /

Name

Panelist Number

Instructions

1 You have presented with 3 Gulf of Mexico Sturgeon samples Please taste the samples in the order presented from left to right Two of the samples are identical and one is different select the odd sample of the three (guess if you can t tell a difference) by circling the number

_____ _____ _____

2 Indicate, with a check mark, the degree of difference between the duplicate samples and the odd sample.

Slight _____
Moderate _____
Much _____
Extreme _____

3 Acceptability

Odd sample more acceptable _____
Duplicates more acceptable _____

4 Comments

Figure A-2 Ballot for triangle sensory test (discriminative test)

Sensory Evaluation of Smoked Gulf of Mexico Sturgeon Samples

Name _____ Date / / 98

Instructions

1) Please take a bite of cracker and a sip of water to rinse your mouth before tasting the samples.
2) Taste all three samples from left to right.
3) Place a vertical line on the location, of the line scale, that best indicates your comparison of each sample according to the given attribute (see example below).
4) Feel free to add any comments at the bottom of this sheet

Thank you! ☺

Example on how to use an unstructured interval scale
Comparison of the degree of lightness between any given samples coded **X**, Y and Z

IMPORTANT: If you have any questions regarding the use of the given scale, PLEASE ask for assistance BEFORE starting the evaluation.

Scales of Attributes for Smoked Sturgeon Samples

General Comments:

Figure A-3 Ballots of descriptive sensory evaluation of smoked sturgeon samples

Sensory Evaluation of Gulf of Mexico Sturgeon

Name _____ Date: / /99

Instructions:

1) Please take a bite of cracker and a sip of water to rinse your mouth before tasting the samples
2) Taste all three samples from left to right
3) Place a vertical line on the location, of the line scale, that best indicates your comparison of each sample according to the given attribute (see example below)
4) Feel free to add any comments at the bottom of this sheet

Thank you! ☺

Example on how to use an unstructured interval scale

Comparison of the degree of lightness between any given samples X, Y and Z

Scales of Attributes for Sturgeon Samples

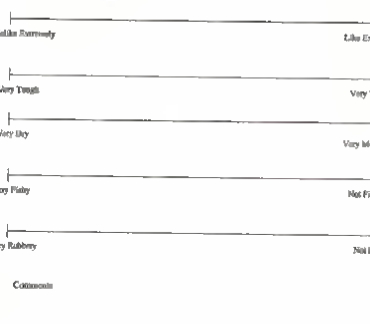

Figure A-4 Ballots of descriptive sensory evaluation of steamed sturgeon samples

APPENDIX B
VIDEO FRAMES OF RIGOR MORTIS STUDY IN GULF STURGEON

Figure B-1 Original video frames of fish GBS

Figure B-2. Original video frames of fish PBS

Figure B 3 Blow up of part of interest from original video frames of fish WSS

Figure B-4 Blow up of part of interest from original video frames of fish GSS

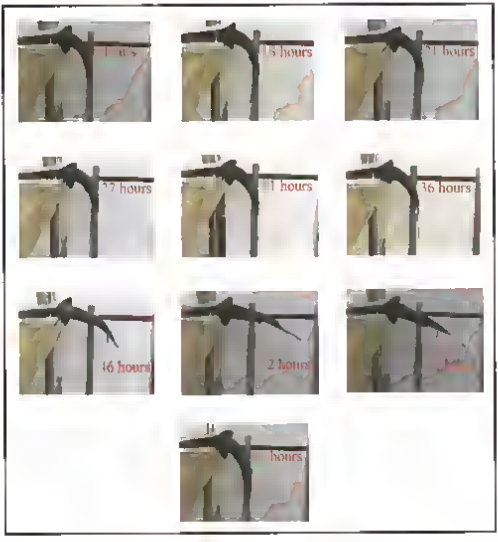

Figure B-5 Blow up of part of interest from original video frames of fish PSS

Figure B-6 Original video frames of fish RBF

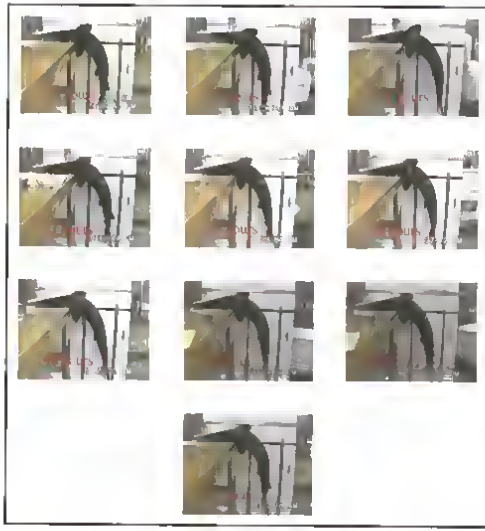

Figure B-7 Original video frames of fish OBF

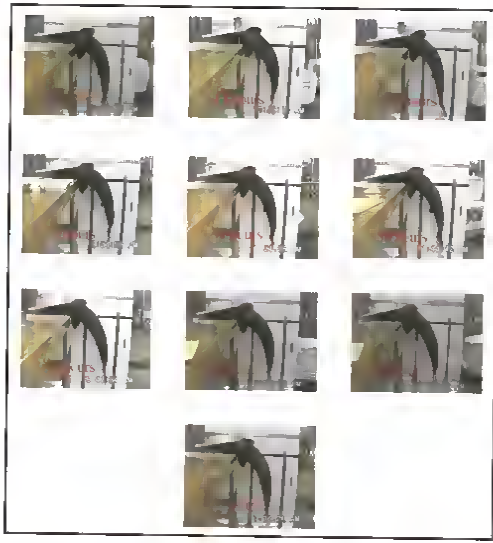

Figure B-8 Original video frames of fish YBF

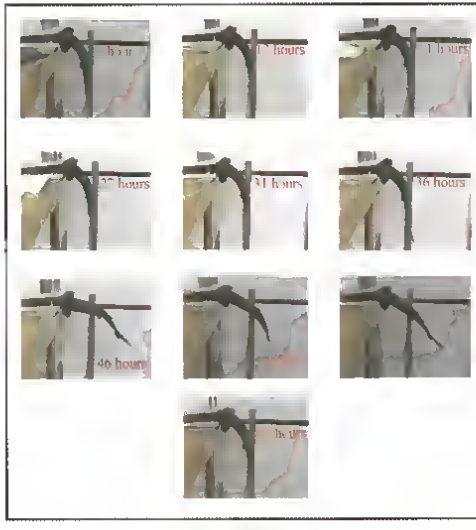

Figure B-9 Blow up of part of interest from original video frames of fish YSF

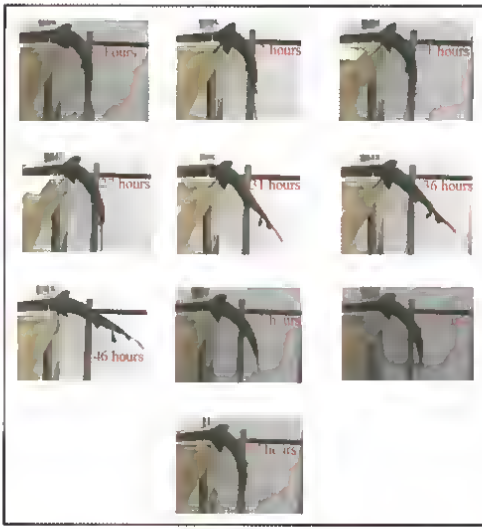

Figure B-10 Blow up of part of interest from original video frames of fish RSF

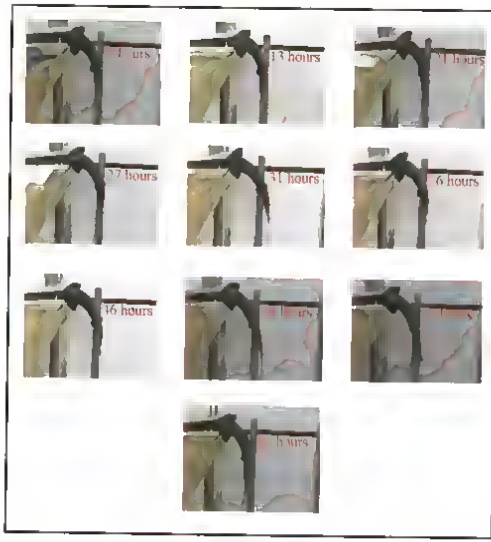

Figure B-11 Blow up of part of interest from original video frames of fish OSF

APPENDIX C
COLOR ANALYSIS RESULTS

Figure C-1 Picture frames of center slices of raw fillets from Gulf sturgeon fed catfish diet

Fish G day 0
Fish H day 0
Fish I day 0
Fish J day 0
Fish K day 0
Fish G day 5
Fish H day 5
Fish I day 5
Fish J day 5
Fish K day 5
Fish G day 10
Fish H day 10
Fish I day 10
Fish J day 10
Fish K day 10
Fish G day 15
Fish H day 15
Fish I day 15
Fish J day 15
Fish K day 15

Figure C-2 Picture frames of center slices of raw fillets from Gulf sturgeon fed bass diet

Fish M day 0
Fish N day 0
Fish P day 0
Fish Q day 0
Fish R day 0
Fish M day 5
Fish N day 5
Fish P day 5
Fish Q day 5
Fish R day 5
Fish M day 10
Fish N day 10
Fish P day 10
Fish Q day 10
Fish R day 10
Fish M day 15
Fish N day 15
Fish P day 15
Fish Q day 15
Fish R day 15

Figure C-3 Picture frames of center slices of raw fillets from Gulf sturgeon fed trout diet

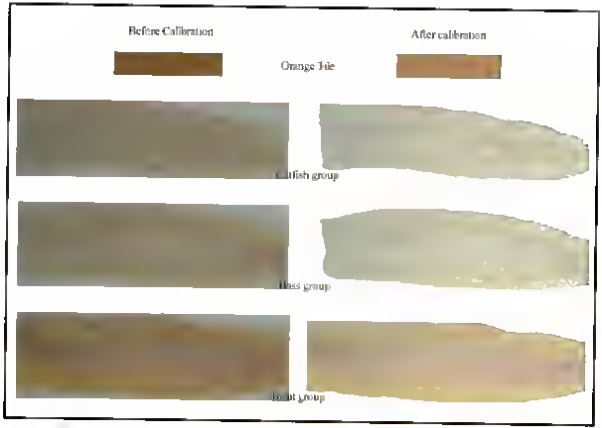

Figure C-4 Picture frames of fillets of Gulf sturgeon fed three different commercial diets before and after calibration

Table C-1 Lab values of center slices fillets from Gulf sturgeon fed three different commercial diets measured with machine vision

Treatment	Fish Code	Storage Days	L	a	b
Catfish	A	0	75 70	7 70	12.51
Catfish	A	5	78 39	5 55	12.63
Catfish	A	10	78 09	6 99	11 00
Catfish	A	15	79 99	4 60	13 17
Catfish	B	0	78 74	5 50	11 18
Catfish	B	5	80 43	5 29	12 49
Catfish	B	10	79 62	8 73	14 42
Catfish	B	15	80 47	4.88	11 43
Catfish	C	0	77 01	7 46	10.58
Catfish	C	5	78 52	6 80	12.72
Catfish	C	10	78.26	9 73	8 87
Catfish	C	15	79.25	7 53	10 96
Catfish	D	0	75 91	10.17	10 03
Catfish	D	5	77 77	6 63	15 00
Catfish	D	10	78 58	6.39	15 54
Catfish	D	15	78 58	8.00	12.26
Catfish	E	0	76 67	8 42	10 63
Catfish	E	5	76 97	8 09	13.33
Catfish	E	10	77 99	8 44	12 82
Catfish	E	15	79 21	7 63	13 00
Bass	G	0	77 39	6 62	8 97
Bass	G	5	78 62	6 71	13 99
Bass	G	10	78 83	7.50	11 52
Bass	G	15	79 79	6 60	10 68
Bass	H	0	79 32	5 99	12.56
Bass	H	5	79 72	7 13	15 86
Bass	H	10	80 27	7 62	15.33
Bass	H	15	81 40	7.27	13 70
Bass	I	0	78 05	6 90	8 53

Table C-1 continued

Treatment	Fish Code	Storage Days	L	a	b
Bass	I	5	78 96	6 00	13 53
Bass	I	10	80 53	8 76	9 75
Bass	I	15	80 60	6 86	11 78
Bass	J	0	77 02	7 07	12 42
Bass	J	5	78 59	8 48	14 41
Bass	J	10	78 72	11 68	9 04
Bass	J	15	79 57	7 52	14 33
Bass	K	0	77 79	6 67	8 08
Bass	K	5	78 59	6 98	12 41
Bass	K	10	79 85	9 71	8 88
Bass	K	15	80 65	5 45	12 75
Trout	M	0	75 76	10 67	10 10
Trout	M	5	76 87	9 86	16 30
Trout	M	10	77 83	10 17	14 06
Trout	M	15	78 53	8 72	14 76
Trout	N	0	73 82	15 64	21 63
Trout	N	5	75 59	12 30	27 73
Trout	N	10	75 94	14 35	25 25
Trout	N	15	79 14	11 11	22 50
Trout	P	0	75 26	11 76	30 70
Trout	P	5	76 98	10 59	34 58
Trout	P	10	78 25	10 96	32 84
Trout	P	15	80 27	8 61	31 69
Trout	Q	0	74 65	11 51	11 42
Trout	Q	5	75 63	11 22	14 39
Trout	Q	10	77 40	11 93	15 36
Trout	Q	15	78 14	9 97	14 33
Trout	R	0	76 82	7 40	20 74
Trout	R	5	77 45	6 60	28 47
Trout	R	10	79 42	6 56	26 78
Trout	R	15	79 47	6 23	25 88

Table C-2 Lab values of whole fillets from Gulf sturgeon fed three different commercial diets measured with machine vision

Treatment	Fish Code	Storage Days	L	a	b
Catfish	A	0	78.76	-4.84	14.54
Catfish	A	5	79.51	-6.42	12.84
Catfish	A	10	79.87	-4.96	11.57
Catfish	A	15	81.38	6.69	13.99
Catfish	B	0	81.82	-5.74	13.51
Catfish	B	5	81.62	-6.73	13.53
Catfish	B	10	81.78	-4.91	16.11
Catfish	B	15	82.58	-7.24	13.55
Catfish	C	0	79.74	5.13	11.81
Catfish	C	5	79.86	5.52	12.96
Catfish	C	10	80.44	-3.43	10.18
Catfish	C	15	81.65	5.07	12.90
Catfish	D	0	77.81	-1.85	12.40
Catfish	D	5	78.63	5.61	17.06
Catfish	D	10	79.64	-5.50	16.72
Catfish	D	15	79.44	-3.22	14.19
Catfish	E	0	79.36	-4.07	13.34
Catfish	E	5	79.00	-4.99	15.09
Catfish	E	10	80.14	-4.18	14.47
Catfish	E	15	80.92	-5.08	14.52
Bass	G	0	81.25	-5.62	12.40
Bass	G	5	80.62	16.11	16.15
Bass	G	10	82.04	-5.90	14.52
Bass	G	15	82.00	5.11	13.67
Bass	H	0	81.67	-6.83	15.61
Bass	H	5	80.34	-5.95	16.60
Bass	H	10	81.68	-5.82	18.10
Bass	H	15	82.34	-6.53	16.11
Bass	I	0	80.77	-5.56	11.03

Table C-2 continued

Treatment	Fish Code	Storage Days	L	a	b
Bass	I	5	80.10	-6.30	13.81
Bass	I	10	81.62	-4.61	11.11
Bass	I	15	82.04	-5.91	12.06
Bass	J	0	79.79	-4.36	14.39
Bass	J	5	79.23	-4.07	16.41
Bass	J	10	79.90	-2.70	11.12
Bass	J	15	80.88	-4.69	16.36
Bass	K	0	80.35	-4.81	10.85
Bass	K	5	80.09	-4.85	13.41
Bass	K	10	80.70	-4.30	9.68
Bass	K	15	82.09	-6.39	13.81
Trout	M	0	78.36	-1.60	12.72
Trout	M	5	77.93	-2.57	17.32
Trout	M	10	79.70	-3.73	16.75
Trout	M	15	80.35	-3.37	16.94
Trout	N	0	75.82	-1.98	25.77
Trout	N	5	76.48	-2.30	31.73
Trout	N	10	77.20	-0.52	28.47
Trout	N	15	79.00	-2.03	26.32
Trout	P	0	77.56	-2.66	35.30
Trout	P	5	77.63	-3.37	37.84
Trout	P	10	78.96	-2.80	36.57
Trout	P	15	81.30	-3.93	35.79
Trout	Q	0	76.67	-2.17	13.06
Trout	Q	5	76.45	-2.60	15.34
Trout	Q	10	78.32	-2.75	16.92
Trout	Q	15	79.18	-3.26	15.70
Trout	R	0	79.19	-5.27	30.36
Trout	R	5	78.66	-5.42	31.78
Trout	R	10	80.97	-5.61	31.76
Trout	R	15	81.18	-4.90	31.45

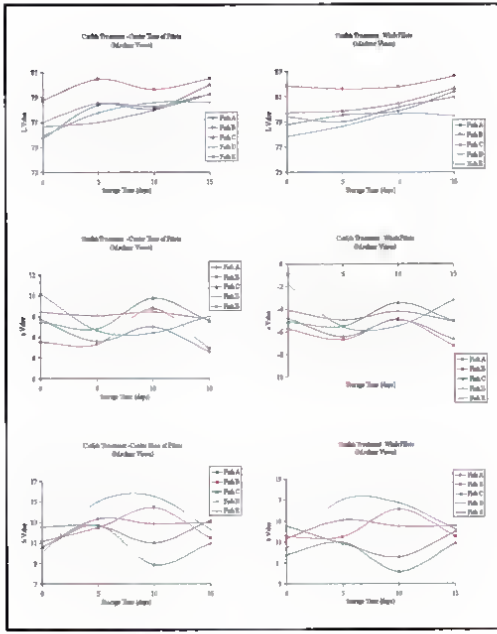

Figure C-5. Machine vision Lab results of center slices and whole fillets of Gulf sturgeon fed catfish diet

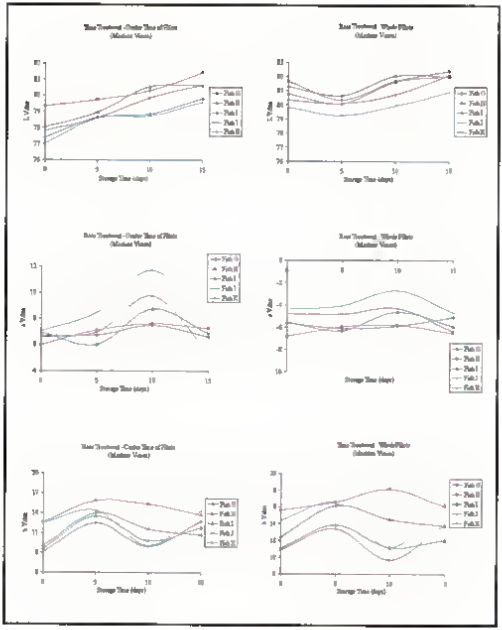

Figure C-6 Machine vision Lab results of center slices and whole fillets of Gulf sturgeon fed bass diet

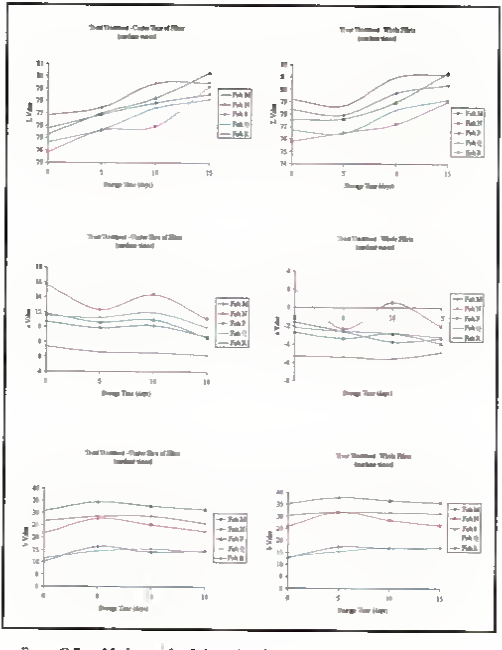

Figure C-7 Machine vision Lab results of center slices and whole fillets of Gulf sturgeon fed trout diet

Table C-3 Lab values of fillets from Gulf sturgeon fed catfish diet measured with CR 200 Chroma Meter

Fish	Day 0			Day 5			Day 10			Day 15		
Code	L	a	b	L	A	B	L	A	b	L	a	b
A1	59 6	11 5	-15 3	60 9	2 2	-2 1	63 7	-2 1	-0 9	64 5	-2 2	-0 6
A2	59 4	11 2	-15 5	55 9	-1 4	-2 3	63 9	-1 9	-0 1	64 8	-2 4	1 0
A3	57 6	12.0	-13 9	59 6	-0 9	-0 3	62 5	-1 3	-0 1	62 9	-2 6	-0 4
Mean Fish A	58 9	11 6	-14 9	58 8	-1 5	-1 6	63 4	-1 8	-0 4	64 1	-2 4	-0 7
SD Fish A	1 4	0 4	1 0	0 9	0 9	1 3	0 8	0 6	0 6	1 1	0 3	0 1
B1	63 6	11 7	-15 2	61 0	-2 2	0 9	62 4	-1 5	-1 4	63 8	-2 7	-0 2
B2	64 0	12 0	-13 9	65 3	-1 0	0 6	60 9	-1 6	-1 2	67 8	-2 3	2 5
B3	65 2	12.2	-14 3	62 5	1 1	-0 8	60 5	1 6	-0 5	64 3	-2 3	1 7
Mean Fish B	64 3	12 0	14 5	62 9	1 4	0 2	61 3	-1 6	-1 0	65.3	-2 4	1 3
SD Fish B	1 1	0 4	0 6	1 1	0 8	1 2	1 3	0 1	0 6	0 4	0 3	1 3
C1	57 0	10 8	-15 6	61 8	-1 5	-0 9	63 1	-1 1	-0 2	62 4	2 2	-0 4
C2	55 4	10 5	-15 0	59 0	-1 4	-1 3	59 0	-1 7	-2 0	61 9	-1 7	-0 6
C3	57 2	11 3	-14 4	59 2	-0 9	0 3	61 2	-1 8	-1 2	63 7	-1 6	-1 3
Mean Fish C	56 5	10 9	-15 0	60 0	-1 3	-0 6	61 1	-1 5	-1 1	62 7	-1 8	-0 8
SD Fish C	0 1	0 4	0 8	1 8	0 4	0 8	1 3	0 5	0 7	0 9	0 4	0 6
D1	60 2	11 6	-14 7	66 2	1 2	1 0	66 5	-1 1	1 7	66 0	-1 0	2 7
D2	65 3	11 5	-14 5	67 2	1 2	1 2	64 5	-1 2	0 5	63 1	-1 0	2 3
D3	56 2	13 2	-14 4	59 5	-1 0	0 3	57 5	0 2	0 8	62 4	-0 3	1 4
Mean Fish D	60 6	12.1	-14 5	64 3	-1 1	0 8	62 8	-0 7	1 0	63 8	-0 8	2 1
SD Fish D	2 8	1 1	0 2	4 7	0 1	0 5	6 4	0 9	0 6	2 5	0 5	0 9

Table C-3 continued

Fish Code	Day 0			Day 5			Day 10			Day 15		
	L	a	b	L	a	b	La	a	b	L	a	b
E1	64 2	11.6	-13 2	58 3	1 1	3.0	63 3	-1 7	1 8	62 8	-1 8	0 1
E2	59 7	11 5	-14 5	58 5	-0 8	-1 4	63 3	-0 9	0 1	61 6	-1.2	0 7
E3	58 2	12 0	-14 4	61 7	-0 8	0 0	66 2	-1 4	0 8	59 8	-1 7	0 2
Mean Fish E	60 7	11 7	-14 0	59 5	-0 2	0 5	64 3	-1 3	0 9	61 4	-1 6	0 3
SD Fish E	4 2	0 3	0 8	2.4	1.3	2 1	2.1	0.2	0 7	2 1	0 1	0 1
Mean Catfish	60.2	11.6	-14.6	61 1	-1 1	-0 1	62.6	-1 4	-0 1	63 5	-1 8	0 5
SD Catfish	2 8	0 5	0 4	2 4	0 5	1 0	1 4	0 4	1 0	1 5	0 7	1 3

Table C-4 Lab values of fillets from Gulf sturgeon fed bass diet measured with CR 200 Chroma Meter

Fish Code	Day 0			Day 5			Day 10			Day 15		
	L	a	B	L	A	b	L	a	b	L	a	b
G1	55 7	10 6	-17 0	64 0	-3 0	1 2	65 8	1 8	0 3	64 4	2 0	0 5
G2	56 4	10 5	-16 1	63 1	-1 6	0 4	65 3	-0 1	0 0	66 7	-2 7	3 0
G3	54 9	11 2	-15.2	64 3	0 8	1 8	66 3	-1 1	0 4	65.2	-2 0	0 7
Mean Fish G	55 7	10 8	-16 1	63 8	-1 3	1 1	65 8	-1 0	0 2	65 4	-2 2	1 4
SD Fish G	0 6	0 4	1 3	0 2	2 7	0 4	0 4	0 5	0 1	0 6	0 0	0 1
H1	64 1	11 0	17 3	61 5	-1 7	0 9	68 2	-1 1	1 7	66 2	-2 7	1 3
H2	61 5	11 1	15 8	62 8	-1 9	0 0	65 0	-1 3	-0 4	63 4	-2 2	0 9

Table C-4 continued

Fish Code	Day 0			Day 5			Day 10			Day 15		
	L	a	b	L	a	b	L	a	b	L	a	b
H3	55.6	11.0	-15.2	61.2	-1.3	0.2	62.5	0.4	0.6	62.6	-1.7	0.3
Mean Fish H	60.4	11.0	-16.1	61.8	-1.6	0.4	65.2	-0.7	0.6	64.1	-2.2	0.8
SD Fish H	6.0	0.0	1.5	0.2	0.3	0.5	4.0	1.1	0.8	2.5	0.7	0.7
I1	58.2	10.7	-15.1	63.1	-1.6	0.8	65.4	-1.1	1.2	64.8	-2.8	1.2
I2	59.5	10.7	-15.1	60.5	-1.6	0.9	65.6	1.5	1.1	64.0	-2.3	0.7
I3	59.5	11.5	-13.7	61.5	-1.3	1.1	66.8	-0.4	3.0	63.6	-1.8	0.7
Mean Fish I	59.1	11.0	-14.6	61.7	-1.5	0.9	65.9	-1.0	1.8	64.1	-2.3	0.9
SD Fish I	0.9	0.6	1.0	1.1	0.2	0.2	1.0	0.5	1.3	0.8	0.7	0.4
J1	55.7	10.7	-15.1	60.6	-2.3	0.7	61.8	-2.3	1.0	65.3	-1.8	1.1
J2	59.5	10.7	-15.1	63.7	-2.1	-0.6	64.4	-1.7	-0.7	64.2	-2.5	0.4
J3	59.5	11.5	-13.7	58.3	-1.5	1.6	61.4	-1.5	-0.7	65.8	-2.1	0.8
Mean Fish J	58.2	11.0	-14.6	60.9	-2.0	0.6	62.5	-1.8	-0.8	65.1	-2.1	0.8
SD Fish J	2.7	0.6	1.0	1.6	0.6	0.6	0.3	0.6	0.2	0.4	0.2	0.2
K1	57.5	9.7	-13.2	62.2	-2.2	-0.2	62.7	-1.4	1.7	64.0	-2.3	0.2
K2	58.5	10.3	-14.5	62.0	-2.0	-0.6	65.1	-2.1	0.5	63.9	-1.9	1.2
K3	57.5	10.9	-14.8	61.0	-1.3	0.0	64.3	-2.4	1.4	63.8	-2.1	1.5
Mean Fish K	57.8	10.3	-14.2	61.7	-1.8	-0.3	64.0	-2.0	1.2	63.9	-2.1	1.0
SD Fish K	0.0	0.8	1.1	0.8	0.6	0.1	1.1	0.7	0.2	0.1	0.1	0.9
Mean Bass	58.2	10.8	-15.1	62.0	-1.6	0.5	64.7	-1.3	0.6	64.5	-2.2	1.0
SD Bass	1.7	0.3	0.9	1.1	0.3	0.5	1.4	0.6	1.0	0.7	0.1	0.3

Table C-5 Lab values of fillets from Gulf sturgeon fed trout diet measured with CR-200 Chroma Meter

Fish	Day 0			Day 5			Day 10			Day 15		
Code	L	a	b	L	a	B	L	a	b	L	a	b
M1	57.9	8.0	-5.7	56.1	-2.4	3.8	58.6	-2.8	6.1	59.0	-3.2	4.7
M2	51.7	8.6	9.2	56.7	-2.1	3.9	60.1	-3.2	9.4	56.3	-2.3	3.0
M3	52.6	9.1	-9.3	55.5	-1.2	3.3	59.7	-1.5	4.8	61.1	3.2	8.7
Mean Fish M	54.1	8.6	-8.1	56.1	-1.9	3.7	59.5	-2.5	6.8	58.8	-2.9	5.5
SD Fish M	3.7	0.8	2.5	0.4	0.8	0.4	0.8	0.9	0.9	1.5	0.0	2.8
N1	58.9	10.8	-13.9	58.7	-1.4	-0.3	64.4	-0.9	0.9	59.9	-1.8	1.3
N2	56.2	12.6	-9.8	59.7	-1.1	0.4	64.4	-0.9	0.9	60.8	-1.2	2.5
N3	55.1	11.9	-12.5	60.1	-1.1	1.6	61.3	-0.3	1.6	61.7	-1.2	2.4
Mean Fish N	56.7	11.8	-12.1	59.5	-1.2	0.6	63.4	-0.7	1.1	60.8	-1.4	2.1
SD Fish N	2.7	0.8	1.0	1.0	0.2	1.3	2.2	0.4	0.5	1.3	0.4	0.8
P1	59.5	11.2	-8.7	63.0	-2.6	6.9	66.1	-1.6	12.1	67.5	-1.3	5.4
P2	57.8	11.6	-9.3	60.4	-0.6	4.9	63.5	-0.3	8.1	63.1	-0.6	7.3
P3	51.6	12.3	-9.7	61.8	0.2	5.8	59.4	-0.4	4.1	66.3	-2.1	8.2
Mean Fish P	56.3	11.7	-9.2	61.7	-1.0	5.9	63.0	-0.8	8.1	65.6	-1.3	7.0
SD Fish P	5.6	0.8	0.7	0.8	2.0	0.8	4.7	0.8	5.7	0.9	0.6	2.0
Q1	55.2	9.1	-10.0	67.5	-2.8	3.6	63.1	-1.9	2.2	61.5	-2.6	3.1
Q2	56.5	9.0	-9.3	56.8	-2.3	2.2	60.3	2.0	3.2	61.6	3.4	6.8
Q3	54.8	9.7	-10.5	58.8	-2.7	3.2	61.7	-2.3	3.5	61.8	2.8	4.7
Mean Fish Q	55.5	9.3	-9.9	61.0	-2.6	3.0	61.7	-2.1	3.0	61.6	2.9	4.9
SD Fish Q	0.3	0.4	0.4	6.2	0.1	0.3	1.0	0.3	0.9	0.2	0.1	1.1

Table C-5 continued

Fish Code	Day 0			Day 5			Day 10			Day 15		
	L	a	b	L	a	b	L	a	b	L	a	b
R1	56.3	10.8	-13.0	59.2	-1.2	0.3	60.7	-0.9	1.0	63.6	-1.5	3.2
R2	58.0	10.6	-13.3	59.4	-1.3	0.5	61.4	-0.9	1.4	60.9	-1.7	1.4
R3	50.8	11.3	-12.0	62.0	-0.4	2.6	63.6	-0.3	2.8	62.8	-1.1	2.3
Mean Fish R	55.0	10.9	-12.8	60.2	-1.0	1.1	61.9	-0.7	1.7			
SD Fish R	3.9	0.4	0.7	2.0	0.6	1.6	2.1	0.4	1.3	0.6	0.3	0.6
Mean Trout	55.5	10.4	-5.7	59.7	1.5	2.8	61.9	-1.3	4.1	61.7	-2.1	4.8
SD Trout	1.1	1.5	2.0	2.2	0.7	2.1	1.5	0.9	3.1	2.9	0.9	2.1

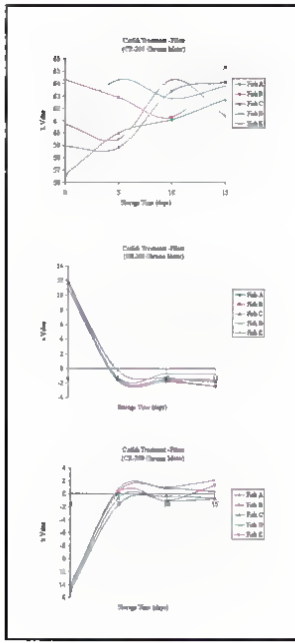

Figure C-8 CR-200 Chroma Meter Lab results of fillets of Gulf sturgeon fed catfish diet

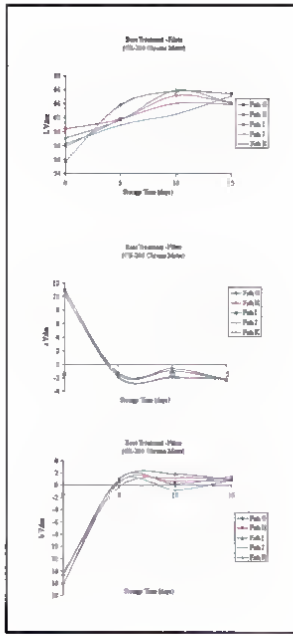

Figure C-9 CR-200 Chroma Meter Lab results of fillets of Gulf sturgeon fed bass diet

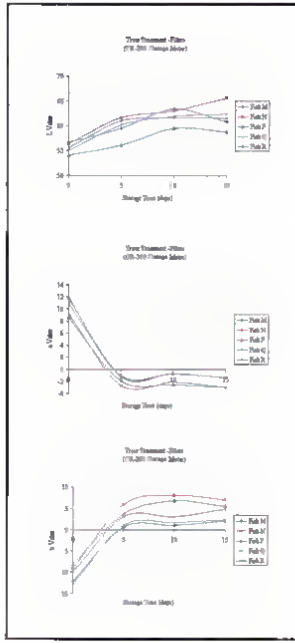

Figure C-10. CR-200 Chroma Meter Lab results of fillets of Gulf sturgeon fed trout diet

APPENDIX D
RESULTS OF FATTY ACID ANALYSIS

Table D-1 Percentage of saturated fatty acids in the extracted oils of diets, raw muscle and smoked muscle of Gulf sturgeons

Sample	12:0	13:0	Iso 14:0	14:0	Iso 15:0	Anti Iso 15:0	15:0	Iso 16:0	16:0	Iso 17:0	17:0	Iso 18:0	18:0	20:0	21:0	22:0	23:0	24:0
Catfish Diet	0.076	0.000	0.000	0.425	0.000	0.000	0.136	0.000	14.914	0.000	0.117	0.050	2.868	0.120	0.000	0.531	0.094	0.140
Catfish Diet	0.000	0.000	0.000	0.427	0.000	0.000	0.117	0.000	14.462	0.000	0.110	0.000	2.619	0.140	0.000	0.783	0.067	0.152
Mean	0.038	0.000	0.000	0.426	0.000	0.000	0.127	0.000	14.688	0.000	0.113	0.025	2.743	0.130	0.000	0.657	0.081	0.146
SD	0.055	0.000	0.000	0.001	0.000	0.000	0.013	0.000	0.320	0.000	0.005	0.035	0.035	0.014	0.000	0.178	0.005	0.009
Bass Diet	0.089	0.000	0.000	4.527	0.196	0.058	0.380	0.006	18.883	0.136	0.385	0.119	3.819	0.458	0.000	0.230	0.000	0.135
Bass Diet	0.110	0.000	0.000	4.456	0.187	0.062	0.390	0.006	18.448	0.121	0.343	0.111	3.696	0.522	0.000	0.252	0.044	0.093
Mean	0.099	0.000	0.000	4.491	0.192	0.060	0.385	0.087	18.665	0.130	0.364	0.115	3.758	0.545	0.000	0.241	0.022	0.114
SD	0.015	0.000	0.000	0.050	0.006	0.003	0.007	0.001	0.308	0.012	0.016	0.006	0.067	0.108	0.000	0.016	0.031	0.030
Trout Diet	0.059	0.030	0.032	4.638	0.194	0.067	0.427	0.070	19.076	0.318	0.378	0.113	4.296	0.064	0.033	0.197	0.047	0.136
Trout Diet	0.070	0.026	0.033	4.966	0.206	0.067	0.417	0.076	19.094	0.241	0.342	0.115	4.352	0.082	0.069	0.204	0.058	0.136
Mean	0.065	0.028	0.032	4.854	0.200	0.067	0.422	0.073	19.085	0.280	0.360	0.114	4.324	0.083	0.066	0.200	0.051	0.136
SD	0.008	0.001	0.001	0.020	0.008	0.000	0.007	0.004	0.013	0.055	0.025	0.001	0.039	0.001	0.047	0.005	0.000	0.000
Raw Catfish	0.026	0.000	0.000	2.277	0.074	0.025	0.311	0.030	18.495	0.077	0.287	0.052	4.305	0.205	0.000	0.000	0.000	0.039
Raw Catfish	0.056	0.000	0.000	2.232	0.073	0.025	0.305	0.028	18.306	0.063	0.261	0.052	4.235	0.209	0.000	0.000	0.000	0.041
Mean	0.041	0.000	0.000	2.254	0.073	0.025	0.308	0.029	18.345	0.070	0.284	0.052	4.270	0.207	0.000	0.000	0.000	0.040
SD	0.022	0.000	0.000	0.032	0.001	0.000	0.005	0.001	0.127	0.010	0.004	0.000	0.050	0.003	0.000	0.000	0.000	0.001
Smoked Catfish	0.020	0.026	0.000	0.526	0.025	0.032	0.191	0.023	18.491	0.023	0.302	0.032	7.744	0.068	0.357	0.065	0.078	0.096
Smoked Catfish	0.060	0.000	0.000	1.602	0.081	0.030	0.258	0.000	16.263	0.049	0.291	0.516	5.946	0.042	0.291	0.000	0.105	0.043
Smoked Catfish	0.000	0.000	0.000	1.351	0.044	0.031	0.218	0.000	16.309	0.036	0.280	0.054	6.618	0.047	0.259	0.266	0.089	0.034

Table D-1 continued

Sample	12-0	13-0	Iso 14-0	14-0	Iso 15-0	Anis Iso 15-0	15-0	Iso 16-0	16-0	Iso 17-0	17-0	Iso 18-0	18-0	20-0	21-0	22-0	23-0	24-0
Mean	0.030	0.000	0.000	1.576	0.052	0.031	0.236	0.000	18.286	0.043	0.288	0.285	9.293	0.045	0.290	0.144	0.097	0.039
SD	0.042	0.000	0.000	0.318	0.012	0.001	0.028	0.000	0.033	0.008	0.008	0.327	0.474	0.004	0.001	0.204	0.011	0.006
Raw Bass	0.048	0.000	0.000	3.048	0.000	0.000	0.125	0.052	18.472	0.134	0.282	0.078	3.549	0.387	0.203	0.000	0.097	0.030
Raw Bass	0.031	0.000	0.000	3.344	0.000	0.000	0.140	0.054	18.882	0.119	0.291	0.077	2.977	0.154	0.208	0.046	0.101	0.029
Raw Bass	0.000	0.000	0.000	2.928	0.113	0.039	0.385	0.051	18.711	0.116	0.288	0.074	3.173	0.249	0.000	0.041	0.094	0.034
Mean	0.028	0.000	0.000	3.108	0.038	0.013	0.207	0.053	18.362	0.123	0.290	0.076	3.233	0.263	0.137	0.029	0.097	0.031
SD	0.023	0.000	0.000	0.215	0.065	0.022	0.126	0.001	0.788	0.010	0.002	0.002	0.290	0.117	0.118	0.025	0.003	0.003
Smoked Bass	0.038	0.000	0.000	2.405	0.082	0.036	0.313	0.039	18.143	0.090	0.323	0.076	5.318	0.120	0.218	0.045	0.108	0.043
Smoked Bass	0.000	0.000	0.000	2.400	0.097	0.038	0.312	0.049	18.111	0.087	0.341	0.072	5.555	0.156	0.211	0.000	0.142	0.039
Smoked Bass	0.023	0.000	0.000	2.329	0.092	0.041	0.309	0.041	18.338	0.095	0.336	0.074	5.370	0.113	0.220	0.043	0.128	0.045
Mean	0.020	0.000	0.000	2.378	0.094	0.040	0.311	0.042	18.198	0.091	0.333	0.074	5.414	0.130	0.217	0.029	0.127	0.042
SD	0.019	0.000	0.000	0.043	0.003	0.001	0.003	0.004	0.254	0.004	0.009	0.002	0.125	0.023	0.005	0.025	0.017	0.003
Raw Trout	0.000	0.000	0.000	2.761	0.085	0.000	0.348	0.044	13.248	0.084	0.332	0.135	5.109	0.150	0.197	0.000	0.082	0.050
Raw Trout	0.000	0.000	0.000	3.396	0.129	0.051	0.368	0.047	18.308	0.000	0.366	0.130	4.013	0.109	0.204	0.000	0.092	0.048
Raw Trout	0.000	0.000	0.000	3.582	0.143	0.033	0.415	0.052	18.770	0.180	0.388	0.115	3.848	0.058	0.228	0.044	0.088	0.048
Mean	0.000	0.000	0.000	3.240	0.122	0.028	0.377	0.048	18.309	0.088	0.355	0.127	4.324	0.118	0.209	0.015	0.087	0.048
SD	0.000	0.000	0.000	0.422	0.025	0.041	0.034	0.004	0.436	0.090	0.020	0.011	0.895	0.032	0.021	0.025	0.005	0.002
Smoked Trout	0.018	0.018	0.000	3.654	0.136	0.044	0.415	0.085	18.889	0.000	0.321	0.090	3.473	0.120	0.250	0.000	0.108	0.052
Smoked Trout	0.022	0.000	0.000	3.552	0.134	0.049	0.420	0.093	20.159	0.183	0.322	0.088	3.358	0.147	0.227	0.090	0.113	0.028
Smoked Trout	0.052	0.000	0.000	2.740	0.110	0.083	0.358	0.041	19.058	0.115	0.115	0.082	5.211	0.185	0.231	0.094	0.129	0.065
Mean	0.031	0.006	0.000	3.315	0.127	0.082	0.398	0.050	18.935	0.103	0.253	0.087	4.013	0.144	0.236	0.061	0.117	0.048
SD	0.018	0.010	0.000	0.501	0.014	0.010	0.034	0.005	0.239	0.097	0.118	0.004	1.038	0.023	0.012	0.036	0.011	0.018

Table D-2 Percentage of monoene fatty acids in the extracted oils of diets, raw muscle and smoked muscle of Gulf sturgeons

Sample	14:1ω7	14:1ω5	16:1ω9	16:1ω7	16:1ω5	17:1ω8	18:1ω9	18:1ω7	18:1ω5	20:1ω11	20:1ω9	20:1ω7	22:1ω11	22:1ω9	24:1ω9
Catfish Diet	0.000	0.000	0.164	0.608	0.000	0.112	15.657	1.351	0.000	0.199	0.438	0.000	0.163	0.486	0.077
Catfish Diet	0.000	0.000	0.201	0.801	0.000	0.164	16.400	1.140	0.110	0.167	0.423	0.000	0.000	0.516	0.061
Mean	0.000	0.000	0.197	0.803	0.000	0.135	15.675	1.250	0.055	0.168	0.430	0.000	0.082	0.502	0.079
SD	0.000	0.000	0.009	0.006	0.000	0.037	0.394	0.157	0.078	0.001	0.009	0.000	0.115	0.016	0.003
Bass Diet	0.000	0.088	0.475	5.139	0.269	0.488	12.860	2.336	0.238	0.354	1.060	0.175	0.072	0.122	0.258
Bass Diet	0.000	0.076	0.433	5.013	0.275	0.590	12.641	2.274	0.226	0.315	1.028	0.141	0.094	0.152	0.244
Mean	0.000	0.082	0.454	5.075	0.272	0.530	12.750	2.305	0.231	0.335	1.044	0.158	0.083	0.137	0.251
SD	0.000	0.014	0.029	0.088	0.011	0.086	0.155	0.044	0.007	0.028	0.023	0.024	0.015	0.021	0.011
Trout Diet	0.052	0.146	0.426	5.155	0.319	0.230	12.585	2.308	0.224	0.225	1.374	0.156	0.618	0.215	0.314
Trout Diet	0.048	0.138	0.480	5.178	0.323	0.201	12.691	2.240	0.227	0.231	1.365	0.180	0.626	0.170	0.316
Mean	0.050	0.141	0.453	5.170	0.321	0.215	12.643	2.274	0.226	0.228	1.369	0.168	0.621	0.193	0.315
SD	0.003	0.007	0.045	0.008	0.003	0.021	0.055	0.046	0.002	0.004	0.006	0.003	0.008	0.031	0.001
Raw Catfish	0.000	0.048	0.474	2.935	0.134	0.104	16.611	2.691	0.264	0.205	1.052	0.089	0.357	0.138	0.135
Raw Catfish	0.000	0.047	0.487	2.936	0.131	0.102	16.932	2.722	0.277	0.208	1.054	0.082	0.345	0.141	0.141
Mean	0.000	0.047	0.481	2.936	0.133	0.103	16.771	2.707	0.270	0.207	1.053	0.086	0.351	0.139	0.138
SD	0.000	0.000	0.009	0.000	0.002	0.001	0.085	0.021	0.005	0.003	0.001	0.005	0.008	0.004	0.004
Smoked Catfish	0.000	0.080	0.313	0.486	0.102	0.134	18.366	2.077	0.385	0.097	0.724	0.037	0.125	0.080	0.167
Smoked Catfish	0.000	0.055	0.428	2.252	0.103	0.138	18.790	2.935	0.253	0.092	1.033	0.109	0.281	0.092	0.166
Smoked Catfish	0.000	0.047	0.410	1.555	0.097	0.121	17.593	2.486	0.244	0.054	1.216	0.091	0.104	0.000	0.165
Mean	0.000	0.061	0.419	2.064	0.085	0.130	18.187	2.661	0.246	0.059	1.124	0.083	0.183	0.046	0.182
SD	0.000	0.006	0.013	0.281	0.011	0.012	0.638	0.247	0.007	0.005	0.129	0.031	0.111	0.085	0.024

Table D-2 continued

Sample	14.1e7	14.1e8	16.1e6	16.1e7	16.1e8	17.1e8	18.1e6	18.1e7	18.1e8	20.1e11	20.1e6	20.1e7	22.1e11	22.1e9	24.1e9
Raw Bass	0.000	0.057	0.468	4.146	0.189	0.195	19.165	2.936	0.278	0.259	0.978	0.115	0.196	0.128	0.158
Raw Bass	0.000	0.060	0.505	4.783	0.218	0.229	20.088	3.006	0.323	0.291	1.003	0.104	0.171	0.127	0.119
Raw Bass	0.029	0.064	0.500	4.436	0.214	0.224	19.213	3.081	0.308	0.102	1.243	0.123	0.191	0.097	0.121
Mean	0.008	0.060	0.491	4.455	0.208	0.216	19.148	3.008	0.303	0.214	1.075	0.114	0.179	0.117	0.133
SD	0.016	0.003	0.020	0.324	0.015	0.018	0.932	0.071	0.023	0.095	0.146	0.010	0.015	0.017	0.023
Smoked Bass	0.000	0.061	0.407	3.459	0.175	0.185	17.031	3.053	0.255	0.361	0.884	0.095	0.183	0.103	0.119
Smoked Bass	0.029	0.072	0.410	3.336	0.172	0.170	17.506	3.064	0.295	0.270	0.960	0.094	0.163	0.132	0.123
Smoked Bass	0.000	0.052	0.397	3.213	0.190	0.169	17.191	3.138	0.282	0.225	0.849	0.099	0.134	0.092	0.110
Mean	0.009	0.062	0.405	3.336	0.178	0.174	17.243	3.081	0.271	0.286	0.884	0.096	0.160	0.109	0.117
SD	0.016	0.010	0.007	0.123	0.004	0.009	0.242	0.042	0.019	0.068	0.019	0.003	0.025	0.021	0.007
Raw Trout	0.000	0.065	0.442	3.744	0.191	0.180	16.157	2.938	0.240	0.454	0.985	0.122	0.297	0.124	0.105
Raw Trout	0.031	0.061	0.457	4.644	0.229	0.158	16.577	2.969	0.297	0.470	1.105	0.137	0.389	0.117	0.102
Raw Trout	0.000	0.0595	0.4967	4.6628	0.2308	0.2229	16.1961	3.0498	0.2342	0.496	0.983	0.129	0.272	0.1471	0.1091
Mean	0.010	0.072	0.485	4.319	0.212	0.186	16.310	2.985	0.254	0.474	1.018	0.129	0.319	0.128	0.105
SD	0.018	0.006	0.029	0.502	0.029	0.034	0.232	0.089	0.029	0.023	0.078	0.008	0.063	0.018	0.003
Smoked Trout	0.037	0.102	0.000	4.985	0.219	0.226	17.315	2.822	0.259	0.065	1.720	0.138	0.350	0.147	0.135
Smoked Trout	0.000	0.080	0.469	4.587	0.228	0.197	19.414	2.932	0.244	0.102	1.438	0.126	0.251	0.133	0.107
Smoked Trout	0.000	0.087	0.441	3.862	0.195	0.182	18.371	2.978	0.212	0.291	0.894	0.110	0.202	0.106	0.118
Mean	0.012	0.090	0.303	4.315	0.214	0.128	18.367	2.910	0.238	0.153	1.350	0.125	0.268	0.129	0.119
SD	0.021	0.011	0.263	0.568	0.017	0.032	0.973	0.079	0.024	0.094	0.420	0.015	0.075	0.021	0.014

Table D-3 Percentage of diene and triene fatty acids in the extracted oils of diets, raw muscle and smoked muscle of Gulf sturgeons

Sample	18:2n6	18:2n6	20:2 nmid*	20:2n6	22:2n6	16:3n3	18:3n6	18:3n4	18:3n3	18:3n1	20:3n6	20:3n3
Catfish Diet	0.045	44.161	0.000	0.092	0.413	0.056	0.122	0.055	4.030	0.086	0.083	0.000
Catfish Diet	0.000	42.580	0.000	0.085	0.461	0.065	0.000	0.000	3.901	0.000	0.081	0.000
Mean	0.023	43.420	0.000	0.093	0.447	0.060	0.061	0.027	3.966	0.024	0.082	0.000
SD	0.032	1.075	0.000	0.002	0.026	0.001	0.086	0.039	0.092	0.034	0.001	0.000
Bass Diet	0.723	16.137	0.245	0.126	0.000	1.063	0.349	0.264	1.815	0.068	0.097	0.073
Bass Diet	0.683	15.840	0.225	0.117	0.126	1.032	0.340	0.276	1.880	0.081	0.118	0.061
Mean	0.703	15.988	0.235	0.121	0.063	1.048	0.344	0.270	1.908	0.075	0.107	0.067
SD	0.028	0.210	0.014	0.006	0.089	0.022	0.006	0.009	0.021	0.008	0.015	0.006
Trout Diet	1.159	15.561	0.204	0.215	0.023	1.204	0.425	0.273	2.328	0.061	0.110	0.059
Trout Diet	1.126	15.435	0.216	0.137	0.000	1.161	0.427	0.273	2.306	0.000	0.117	0.061
Mean	1.143	15.498	0.210	0.177	0.012	1.183	0.426	0.273	2.315	0.030	0.114	0.060
SD	0.023	0.089	0.008	0.058	0.016	0.016	0.001	0.000	0.015	0.043	0.005	0.001
Raw Catfish	0.226	17.622	0.127	1.603	0.073	0.286	1.187	0.225	1.140	0.051	1.516	0.241
Raw Catfish	0.234	17.705	0.122	1.616	0.086	0.270	1.190	0.229	1.157	0.089	1.516	0.241
Mean	0.230	17.663	0.124	1.605	0.070	0.266	1.185	0.227	1.148	0.089	1.517	0.241
SD	0.006	0.059	0.003	0.006	0.005	0.003	0.026	0.003	0.012	0.006	0.001	0.000
Smoked Catfish	0.086	17.702	0.007	2.800	0.037	0.093	1.285	0.140	0.770	0.106	3.192	0.043
Smoked Catfish	0.167	15.995	0.120	1.774	0.033	0.226	0.858	0.165	0.939	0.060	1.625	0.029
Smoked Catfish	0.140	17.656	0.067	2.043	0.030	0.179	1.190	0.186	0.867	0.075	2.185	0.000
Mean	0.154	16.829	0.104	1.908	0.032	0.202	1.006	0.177	0.853	0.078	1.906	0.015
SD	0.015	1.174	0.023	0.180	0.002	0.034	0.214	0.012	0.020	0.008	0.385	0.021

Table D-3 continued

Sample	16:2ω6	18:2ω6	20:2 nmid[a]	20:2ω6	22:2ω6	16:3ω3	18:3ω6	18:3ω4	18:3ω3	18:3ω1	20:3ω6	20:3ω3
Raw Bass	0.377	12.284	0.176	0.790	0.067	0.374	0.540	0.366	1.125	0.073	0.459	0.056
Raw Bass	0.484	13.772	0.142	0.750	0.038	0.406	0.611	0.428	1.328	0.058	0.481	0.064
Raw Bass	0.412	13.301	0.174	0.881	0.046	0.358	0.524	0.411	1.224	0.071	0.552	0.226
Mean	0.416	13.122	0.164	0.807	0.051	0.380	0.592	0.401	1.226	0.071	0.497	0.116
SD	0.044	0.766	0.018	0.067	0.031	0.026	0.045	0.030	0.102	0.003	0.046	0.098
Smoked Bass	0.314	11.044	0.115	0.818	0.037	0.332	0.437	0.358	0.937	0.065	0.484	0.044
Smoked Bass	0.304	10.574	0.128	0.827	0.046	0.331	0.437	0.348	0.876	0.100	0.515	0.044
Smoked Bass	0.317	11.686	0.138	0.852	0.040	0.340	0.518	0.387	0.954	0.087	0.520	0.044
Mean	0.312	11.103	0.127	0.832	0.042	0.334	0.464	0.354	0.922	0.087	0.506	0.047
SD	0.007	0.559	0.012	0.016	0.006	0.005	0.047	0.006	0.041	0.003	0.020	0.003
Raw Trout	0.384	11.494	0.155	0.567	0.000	0.443	0.376	0.378	1.234	0.086	0.258	0.044
Raw Trout	0.685	13.304	0.177	0.570	0.023	0.571	0.437	0.395	1.558	0.067	0.000	0.081
Raw Trout	0.5533	13.8556	0.167	0.6432	0.026	0.581	0.4947	0.4475	1.662	0.0928	0.2456	0.055
Mean	0.541	12.888	0.166	0.597	0.016	0.532	0.436	0.407	1.485	0.080	0.184	0.050
SD	0.151	1.240	0.011	0.038	0.014	0.077	0.056	0.036	0.223	0.011	0.146	0.005
Smoked Trout	0.662	13.715	0.173	0.658	0.025	0.603	0.587	0.436	1.573	0.076	0.272	0.058
Smoked Trout	0.673	13.067	0.160	0.604	0.028	0.534	0.486	0.395	1.516	0.095	0.232	0.055
Smoked Trout	0.484	10.870	0.133	0.695	0.058	0.458	0.384	0.355	1.186	0.078	0.262	0.055
Mean	0.606	12.484	0.155	0.652	0.037	0.465	0.472	0.395	1.425	0.074	0.258	0.056
SD	0.106	1.504	0.020	0.044	0.018	0.038	0.092	0.041	0.210	0.008	0.021	0.002

[a] non methylene interrupted diene

Table D-4 Percentage of tetraene- pentaene and hexaene fatty acids in the extracted oils of diets, raw muscle and smoked muscle of Gulf sturgeons fed three different commercial diets

Sample	16:4n3*	18:4n3	18:4n1	20:4n6	20:4n3	22:4n6	22:4n3	20:5n3	21:5n3	22:5n6	22:5n3	22:6n3
Catfish Diet	0.000	0.058	2.848	0.205	0.000	0.000	0.000	0.402	0.413	0.076	0.071	0.329
Catfish Diet	0.000	0.060	4.042	0.212	0.061	0.000	0.000	0.426	0.481	0.000	0.073	0.381
Mean	0.000	0.059	3.495	0.208	0.030	0.000	0.000	0.414	0.447	0.038	0.072	0.355
SD	0.000	0.003	0.774	0.005	0.043	0.000	0.000	0.018	0.049	0.054	0.001	0.037
Bass Diet	0.484	1.748	1.143	0.486	0.499	0.094	0.057	6.528	0.325	0.282	1.184	7.638
Bass Diet	0.343	1.750	1.750	0.491	0.491	0.060	0.084	6.724	0.438	0.262	1.133	7.272
Mean	0.404	1.748	1.447	0.488	0.495	0.067	0.065	6.775	0.382	0.272	1.144	7.408
SD	0.086	0.003	0.430	0.006	0.006	0.010	0.002	0.073	0.081	0.014	0.015	0.166
Trout Diet	0.714	1.835	0.453	0.518	0.059	0.090	0.096	7.786	0.536	0.238	1.210	7.060
Trout Diet	0.652	1.842	0.459	0.524	0.000	0.083	0.095	7.886	0.600	0.238	1.293	6.897
Mean	0.683	1.838	0.456	0.520	0.030	0.086	0.092	7.837	0.568	0.237	1.252	7.038
SD	0.044	0.005	0.004	0.008	0.042	0.005	0.004	0.069	0.049	0.002	0.058	0.059
Raw Catfish	0.085	0.514	1.369	1.877	0.585	0.159	0.091	2.750	0.215	0.360	1.359	7.043
Raw Catfish	0.085	0.530	1.252	2.050	0.553	0.160	0.070	2.841	0.238	0.358	1.278	7.182
Mean	0.085	0.522	1.311	2.014	0.569	0.159	0.080	2.815	0.227	0.359	1.319	7.112
SD	0.000	0.011	0.086	0.052	0.008	0.001	0.014	0.036	0.016	0.001	0.056	0.089
Smoked Catfish	0.000	0.232	0.856	4.851	0.000	0.221	0.090	3.600	0.060	0.619	1.468	8.859
Smoked Catfish	0.078	0.269	0.852	2.869	0.485	0.180	0.064	3.876	0.084	0.372	1.614	9.683
Smoked Catfish	0.078	0.375	0.827	3.648	0.437	0.227	0.079	3.427	0.189	0.536	1.519	9.280
Mean	0.077	0.337	0.730	3.456	0.462	0.204	0.072	3.553	0.127	0.454	1.566	9.477
SD	0.001	0.054	0.145	0.876	0.035	0.033	0.015	0.178	0.046	0.116	0.086	0.306

Table D-4 continued

Sample	16:4n3*	18:4n3	18:4n1	20:4n6	20:4n3	22:5n6	22:4n3	20:5n3	21:5n3	22:5n3	22:6n3	22:6n3
Raw Bass	0.086	0.483	2.554	1.211	0.506	0.108	0.104	4.738	0.271	0.292	1.831	8.452
Raw Bass	0.113	0.564	0.847	0.960	0.452	0.102	0.109	4.495	0.284	0.285	1.920	8.514
Raw Bass	0.286	0.492	1.317	1.036	0.568	0.105	0.115	4.723	0.234	0.281	1.936	8.440
Mean	0.166	0.508	1.573	1.070	0.508	0.105	0.109	4.852	0.263	0.286	1.930	8.135
SD	0.108	0.052	0.882	0.128	0.058	0.003	0.005	0.136	0.026	0.008	0.009	0.538
Smoked Catfish	0.076	0.366	0.818	1.870	0.768	0.116	0.108	7.130	0.239	0.338	2.196	12.784
Smoked Catfish	0.070	0.371	1.087	2.037	0.786	0.131	0.104	6.651	0.231	0.354	2.196	12.553
Smoked Catfish	0.078	0.354	0.864	2.082	0.825	0.108	0.103	7.140	0.238	0.388	2.220	12.670
Mean	0.074	0.363	0.923	2.000	0.793	0.118	0.104	6.974	0.235	0.350	2.205	12.669
SD	0.003	0.010	0.093	0.116	0.029	0.013	0.002	0.279	0.004	0.011	0.013	0.116
Raw Trout	0.118	0.636	0.955	1.785	0.748	0.121	0.079	7.565	0.264	0.356	1.882	12.813
Raw Trout	0.158	0.884	0.703	1.496	1.066	0.109	0.102	8.472	0.347	0.308	1.908	11.353
Raw Trout	0.152	0.9507	0.512	1.1748	0.9864	0.108	0.1039	5.879	0.371	0.2967	2.0338	10.272
Mean	0.145	0.790	0.723	1.486	0.940	0.112	0.095	6.639	0.327	0.317	1.935	11.479
SD	0.024	0.223	0.222	0.305	0.173	0.005	0.014	0.858	0.058	0.038	0.088	1.275
Smoked Trout	0.131	0.927	0.838	1.016	0.891	0.114	0.116	5.787	0.373	0.298	1.642	10.078
Smoked Trout	0.163	0.813	0.713	1.074	0.828	0.108	0.108	6.574	0.380	0.311	1.732	10.930
Smoked Trout	0.108	0.926	0.920	1.640	0.715	0.101	0.085	7.613	0.265	0.346	1.948	13.102
Mean	0.134	0.789	0.774	1.311	0.845	0.108	0.103	6.658	0.339	0.318	1.774	11.289
SD	0.027	0.228	0.127	0.458	0.114	0.007	0.016	0.918	0.064	0.026	0.158	1.611

* position of double bond not determined

Table D-5 Summary of results of fatty acid analysis

Sample	% Saturated FAs	% Monoenes	% Dienes	% Trienes	% Tetraenes	% Pentaenes	% Hexaene	% Identified FAs	% Unknown Peaks	% ω3 FAs	% ω6 FAs	Ratio ω3 / ω6
Catfish Diet	19.273	19.793	44.731	4.403	3.208	0.962	0.328	92.698	7.301	5.368	45.217	0.118
Catfish Diet	18.897	19.031	43.235	4.047	4.374	0.982	0.381	90.947	9.053	5.449	43.528	0.125
Mean	19.085	19.412	43.983	4.225	3.791	0.972	0.355	91.823	8.177	5.408	44.373	0.122
SD	0.266	0.538	1.058	0.252	0.824	0.014	0.037	1.239	1.239	0.058	1.194	0.005
Bass Diet	29.465	23.565	17.230	3.833	4.531	8.887	7.538	95.071	4.929	21.231	18.306	1.160
Bass Diet	28.980	23.424	16.991	3.798	4.968	8.558	7.272	94.018	5.981	20.875	18.056	1.156
Mean	29.237	23.845	17.111	3.815	4.750	8.572	7.406	94.545	5.455	21.053	18.181	1.158
SD	0.350	0.312	0.186	0.026	0.323	0.021	0.183	0.744	0.744	0.252	0.177	0.003
Trout Diet	30.466	25.196	17.163	4.461	3.786	9.770	7.080	97.881	2.108	22.190	18.336	1.210
Trout Diet	30.585	25.238	16.915	4.366	3.654	10.017	6.997	97.771	2.229	22.283	18.089	1.231
Mean	30.540	25.201	17.039	4.414	3.705	9.894	7.038	97.831	2.169	22.226	18.212	1.221
SD	0.063	0.049	0.176	0.066	0.072	0.175	0.058	0.085	0.085	0.052	0.176	0.015
Raw Catfish	28.184	27.458	18.651	4.627	4.748	4.724	7.043	94.448	5.556	14.222	24.724	0.575
Raw Catfish	25.905	27.808	18.743	4.823	4.710	4.714	7.182	94.494	5.518	14.359	24.855	0.578
Mean	26.050	27.633	18.697	4.725	4.729	4.719	7.112	94.484	5.536	14.290	24.789	0.576
SD	0.204	0.106	0.066	0.003	0.027	0.007	0.098	0.028	0.028	0.096	0.092	0.002
Smoked Catfish	29.091	21.512	20.412	5.629	5.626	5.747	8.953	96.179	3.824	18.292	30.372	0.603
Smoked Catfish	27.724	29.597	18.083	3.943	4.928	5.759	8.893	96.741	3.259	17.123	23.998	0.714
Smoked Catfish	27.857	24.442	18.859	4.734	5.770	5.840	9.260	97.462	2.538	18.401	27.628	0.667
Mean	27.593	25.520	19.026	4.335	5.349	5.782	9.477	97.102	2.886	19.762	25.993	0.650
SD	0.051	1.523	1.319	0.549	0.585	0.084	0.306	0.510	0.510	0.511	2.780	0.068

Table D-5 continued

Sample	% Saturated FAs	% Monoenes	% Dienes	% Trienes	% Tetraenes	% Pentaenes	% Hexaene	% Identified FAs	% Unknown Peaks	% ω3 FAs	% ω6 FAs	Ratio ω6 / ω3
Raw Bass	28.512	29.289	13.734	2.634	5.341	7.233	6.462	93.553	6.447	18.316	16.167	1.186
Raw Bass	27.534	30.995	16.167	3.367	3.946	6.953	5.814	96.216	3.785	18.637	17.464	1.087
Raw Bass	27.284	29.887	14.618	3.468	4.321	7.177	8.440	95.373	3.827	18.716	17.242	1.144
Mean	27.103	29.720	14.672	3.283	4.436	7.131	8.135	95.381	4.616	18.225	16.958	1.138
SD	0.530	1.356	0.747	0.253	0.853	0.131	0.538	1.584	1.584	0.547	0.684	0.064
Smoked Catfish	28.412	26.351	12.330	2.691	4.243	8.302	12.784	96.713	3.287	24.928	15.460	1.612
Smoked Catfish	28.808	25.877	11.883	2.851	4.586	8.435	12.553	96.403	3.587	24.146	15.228	1.586
Smoked Catfish	27.694	26.136	13.035	2.835	4.554	8.855	12.870	97.082	2.918	24.925	16.492	1.511
Mean	28.305	26.388	12.416	2.726	4.464	8.754	12.569	96.733	3.267	24.536	15.727	1.570
SD	0.366	0.271	0.581	0.097	0.193	0.285	0.118	0.340	0.340	0.451	0.795	0.052
Raw Trout	28.624	28.001	12.600	2.825	4.342	10.048	12.613	97.252	2.748	25.588	15.344	1.668
Raw Trout	27.917	27.616	14.759	3.079	4.535	9.036	11.353	98.295	1.705	24.332	16.932	1.437
Raw Trout	28.896	27.3087	15.2854	3.586	3.898	8.870	10.272	97.967	2.033	22.894	17.408	1.315
Mean	28.506	28.674	14.208	3.197	4.292	9.218	11.479	97.838	2.162	24.272	16.561	1.473
SD	0.584	0.857	1.418	0.378	0.272	0.755	1.275	0.533	0.533	1.348	1.081	0.179
Smoked Trout	28.443	28.224	15.233	3.487	3.885	8.098	10.076	97.446	2.554	21.948	17.327	1.267
Smoked Trout	28.923	27.3051	14.532	3.266	4.007	8.897	10.930	97.660	2.340	23.373	16.563	1.411
Smoked Trout	26.486	24.7944	12.04	2.790	4.266	10.173	13.102	96.859	3.318	25.955	14.840	1.746
Mean	28.955	28.775	13.635	3.178	4.083	9.056	11.269	97.263	2.737	23.758	16.243	1.475
SD	0.529	1.775	1.678	0.362	0.211	1.040	1.611	0.513	0.513	2.032	1.274	0.248

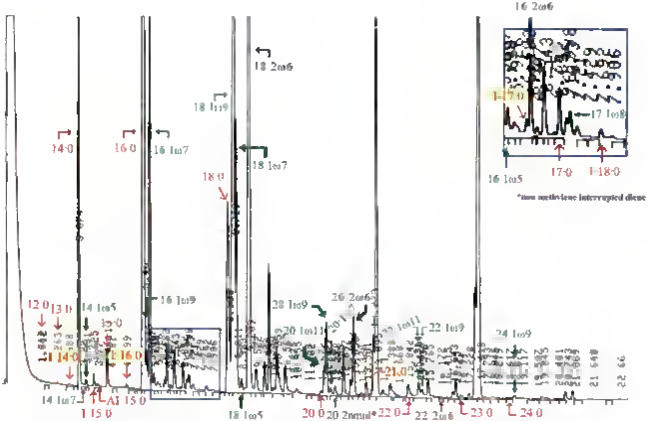

Figure D-1 Cromatogram of the oil extracted from raw muscle of gulf sturgeon fed trout diet showing saturated, monoene and diene fatty acids identified

Figure D-2. Chromatogram of the oil extracted from raw muscle of gulf sturgeon fed trout diet showing triene and tetraene, pentaene and hexaene fatty acids identified

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BIOGRAPHICAL SKETCH

Alexandra Marques de Oliveira was born in 1968, in Rio de Janeiro, Brazil. In 1986 she joined the Chemistry Department at Universidade Federal Fluminense, in RJ, Brazil. During her undergraduate studies, Alexandra was awarded a scholarship from CNPq (Conselho Nacional de Pesquisas) for four subsequent years. Over this period she worked under the supervision of Dr. Gilberto Alves Romeiro investigating possible pathways to the synthesis of heterocyclic compounds with biological activity. In 1990, Alexandra obtained B.S. degree in industrial chemistry. In 1991 she joined the Organic Chemistry Department at Instituto Militar de Engenharia in RJ, Brasil. Under supervision of Dr. Peter Seidl, Alexandra carried on research in the field of molecular structure and odor of monoterpenes. In 1994 she earned her M.S. degree in organic chemistry, and joined the Research and Development Center of Souza Cruz S.A. (RJ, Brazil). She worked at this tobacco company for about a year on the sector of aromas and casing. In August 1995 Alexandra joined the Food Science Department at the University of Florida. In 1997 she became a member of the honor society of Agriculture, Gamma Sigma Delta. Over the last five years she has been a teaching assistant in food chemistry, food analysis and advanced lipid chemistry. Alexandra plans to find a position in the food industry to work in the field of food chemistry after completion of her degree.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate in scope and quality as a dissertation for the degree of Doctor of Philosophy

Sean Francis O Keefe, Chair
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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate in scope and quality, as a dissertation for the degree of Doctor of Philosophy

Murat O Balaban, Cochair
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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

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This dissertation was submitted to the Graduate Faculty of the College of Agricultural and Life Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy

May 2001

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